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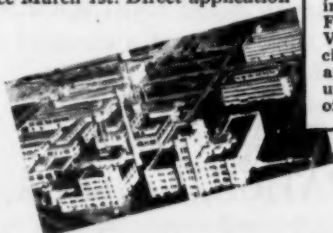
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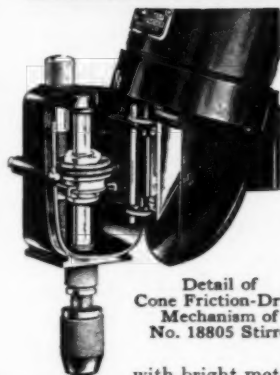
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EXPERIMENTAL DESIGN FOR CEREAL CHEMISTS

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Dominion Rust Research Laboratory, Winnipeg, Manitoba

(Presented at the Annual Meeting, 1942; received for publication January 12, 1944)

In the application to experiments of the principles of statistics, there is no clear line of demarcation between design and analysis. In order to think clearly about the design of a particular experiment it is necessary to have in mind the method of analysis that is to be used. Similarly, a study of different methods of analysis will suggest different designs. It is with this point in mind that certain suggestions are made in this paper that may be of value to experimentalists in cereal chemistry.

The intimate relation between experimental design and method of analysis is well illustrated by examples in which the end results are to be expressed in terms of correlation and regression. It is perhaps too frequently assumed that, in order to determine such relationships with a reasonable degree of accuracy, it is necessary to have a fairly large number of pairs of values in a homogeneous population. In order to illustrate this point, let us suppose that we are to determine the relation between the total nitrogen content of barley and the saccharifying activity of the malt extract expressed in degrees Lintner. In order to obtain a reliable measure of a correlation it is true that we should have a fairly large number of pairs of values, but it is not true that we must obtain these values from one variety or all from samples grown in one area. Methods of analysis are now available that enable us to group the results for a series of varieties and for samples of these varieties obtained in several areas. As a matter of fact, any determination of the correlation from one variety or from samples grown under a limited range of environmental conditions may be a pure waste of time. If different varieties give different correlations, it is perfectly clear that an experiment with one variety will not answer the question. Further, it seems quite possible that if the samples are all grown under conditions that give a uniform protein level, the relation determined may not be representative of the relation that actually exists in samples grown

under a wide range of climatic and soil conditions and giving a wide range of protein levels.

The same general principles should be kept in mind in almost any experiment. The correlation between protein and loaf volume might well be studied under different baking conditions, with different formulas, with different levels of the ingredients of the mix, and with different time elements entering into parts of the procedure. It is not desirable to include too many factors, as the experiment may become huge, unwieldy, and extremely complex; but it is always worth while to make a careful study of the various factors that might affect the results, and plan to vary at least those factors that are known from general experience to be important.

Experiments with more than one factor at different levels are usually referred to as factorial experiments. A great deal has been written on such experiments, especially in connection with field plot trials, and a considerable number of them may be found described in the literature of cereal chemistry. However, when two or more variables are involved in the study which otherwise is essentially similar to the factorial experiment, there is apparently a more limited knowledge with respect to appropriate methods of analysis. The procedure is generally known as the covariance analysis. One of the objects of this paper is to present this method by means of examples taken from cereal chemistry, and to illustrate where possible how a knowledge of the method may have a bearing on experimental design.

Covariance Analysis

The covariance technique is well illustrated in a study conducted by Anderson *et al* (1939) on the correlation between total nitrogen and saccharifying activity of the malt extracts for 12 varieties of barley grown at 12 stations distributed across Canada. The 12 varieties were those most commonly grown in Canada, and there was at least one station in each of the important barley-growing districts.

The experiment provided 144 pairs of values of the two variables. If these are set up in the form of a scatter diagram, the scatter is considerable and does not indicate a very high value of the correlation coefficient. The actual value is 0.694. However, the material is very heterogeneous; and since part of the scatter may be due to this heterogeneity, the next step in the procedure is to determine the correlations for those components of the experiment that can be considered homogeneous. For example, we can determine a correlation coefficient for each variety, for the variety means, for the station means, or for all varieties combined after eliminating the effect of variety means and station means. The correlation coefficients determined for each va-

riety will be expected to show a good deal of variability, especially since they will be determined from only 12 pairs of values. It will be important, therefore, to make a test of the variation in these correlations. The procedures are illustrated below.

In the first place, we carry out the calculations necessary for setting up a preliminary analysis of covariance table, from which we can calculate some of the correlation and regression coefficients. These data are summarized in Table I, in which total nitrogen is represented by x

TABLE I
PRELIMINARY ANALYSIS OF COVARIANCE

	$\Sigma(x^2)$	$\Sigma(xy)$	$\Sigma(y^2)$	DF	Re- gres- sion	Residual	DF	r_{xy}	b_{yz}
Stations	18.1587	22.4636	30.1980	11	27.7891	2.4089	10	.959	1.2371
Varieties	0.8672	-0.09620	19.2276	11	0.0107	19.2169	10	-.024	-0.1109
Error	1.1296	1.4189	8.8040	121	1.7823	7.0217	120	.450	1.2561

and saccharifying activity by y ; x and y are measured in terms of deviations from their respective means, that is, in the table, $\Sigma(x^2) = \Sigma(x - \bar{x})^2$, $\Sigma xy = \Sigma(x - \bar{x})(y - \bar{y})$, and $\Sigma(y^2) = \Sigma(y - \bar{y})^2$. The sums of squares of x and y are calculated as in the analysis of variance. The sums of products are calculated by an exactly analogous procedure. For example, the sum of products for stations is obtained by multiplying corresponding station totals for x and y and summing. This sum is divided by 12, the number of individual determinations entering into each total, and the correction term is found by multiplying the totals for x and y and dividing by 144.

The degrees of freedom in the 5th column represent the sums of squares of x and y . In the column headed "regression" we have that portion of the sum of squares of y that is accounted for by the linear regression. It is most easily calculated from $[\Sigma(xy)]^2/\Sigma(x^2)$, the values of $\Sigma(xy)$ and $\Sigma(x^2)$ being taken from the same line. From the sets of sums of squares and products we can calculate the correlation and regression coefficients. Thus—

$$r_{xy} = \frac{\Sigma(xy)}{\sqrt{\Sigma(x^2)\Sigma(y^2)}} \quad \text{and} \quad b_{yz} = \frac{\Sigma(xy)}{\Sigma(x^2)}$$

The "residual" is obtained by subtracting the sum of squares for regression from the sum of squares for y in the same line. Since each regression sum of squares represents one degree of freedom, the degrees of freedom given in the 8th column of the table are in all cases one less than the degrees of freedom for the original sums of squares of y . It is not necessary to calculate the 6th, 7th, and 8th columns in order

to determine the correlation and regression coefficients, but these columns are necessary for certain tests that we may wish to apply.

The most striking feature of the analysis so far is the wide differences between the correlations for the station means and the variety means. Also, there is an evident relation between the total nitrogen and saccharifying activity as the total nitrogen changes from station to station. Although the station correlation is represented by only 10 degrees of freedom, it is highly significant. The variety correlation is quite insignificant. This indicates that genetic factors that cause differences between the varieties in the total nitrogen content of the grain do not have a corresponding effect on the saccharifying activity of the malt extract. This is an excellent example of the heterogeneity of the covariance and illustrates clearly the necessity for the separation of the total covariance into its component parts.

The next point to clear up in this study is the variation in the regressions for individual varieties. We know that when the varieties are considered as a group, the correlation between the two variables is high. This is indicated by the value of the coefficient for the station means. This correlation may be significantly higher for some varieties than for others, and this will be important information for us to have in evaluating the varieties. A test of the significance of the heterogeneity of the variety regressions can be carried out very easily by means of an extension of the covariance analysis.

The first step is to calculate the sums of squares and products for each variety. These are given in Table II.

TABLE II
ANALYSIS OF COVARIANCE FOR TESTING HETEROGENEITY OF VARIETY REGRESSIONS

Variety	$\Sigma(x^2)$	$\Sigma(xy)$	$\Sigma(y^2)$	DF	Regression	Residual	DF
1	1.1498	1.7903	2.9745	11	2.7876	0.1869	10
2	1.8104	0.9613	0.8930	11	0.5104	0.3826	10
3	1.6141	1.8985	3.9864	11	2.2330	1.7534	10
4	1.2609	1.9195	3.2895	11	2.9221	0.3674	10
5	1.5873	1.7014	2.1109	11	1.8237	0.2872	10
6	1.7591	2.6461	4.6605	11	3.9804	0.6801	10
7	1.6923	2.7206	4.7231	11	4.3737	0.3494	10
8	1.8202	2.9503	5.1203	11	4.7820	0.3383	10
9	1.7302	1.5772	1.7513	11	1.4377	0.3136	10
10	2.0446	3.4896	6.6857	11	5.9558	0.7299	10
11	1.3582	1.1139	1.5959	11	0.9135	0.6824	10
12	1.4622	1.1139	1.2111	11	0.8486	0.3625	10
Column totals	19.2893	23.8826	39.0022	132		6.4337	120
All varieties	19.2893	23.8826	39.0022	132	29.5697	9.4325	131

In each line including the last, the regression sum of squares is calculated as in Table I. The test of significance is finally as follows—

	Residual	DF	Mean square	F	5% pt
All varieties	9.4325	131			
Within varieties	6.4337	120	0.05361		
Difference	2.9988	11	0.2726	5.08	1.86

The F value is high and definitely establishes the significance of differences among the regressions for the varieties.

There are other procedures and tests of significance that flow from the covariance analysis, but those described in this example are undoubtedly of the greatest importance for experiments of this type. We shall now discuss a type of experiment in which the covariance analysis is applied in a somewhat different manner.

Subdivision of Treatment Components

A fairly common type of experiment is one in which a number of treatments are compared at two or more levels of a second factor. For example, we might have a series of flours of different varieties that are to be tested for loaf volume with the addition of different amounts of potassium bromate. A study of published results indicates that a method of analysis particularly appropriate for data from experiments of this kind is rarely used. A greater knowledge of this method would seem to be worth while in the extraction of the maximum amount of information from the experiment, and it also seems likely that a knowledge of the technique of analysis would be of assistance in working out the design. Foreknowledge of what one is going to do with data is bound to have some influence on how the experiment is laid out.

In order to demonstrate the method of analysis it will be necessary to illustrate the procedure known in statistics as the splitting up of degrees of freedom into orthogonal components. If we have three determinations that we shall designate by A, B, and C, the differences between these three determinations are represented by two degrees of freedom. The two degrees of freedom can be split up into orthogonal components. In the sense in which it is used here, the word "orthogonal" means independent. In other words, one of the components can have any value whatever without its having any effect on the value of the other component. One of the components can be defined arbitrarily, but when the first one is defined the second one is fixed by rule. Three simple ways of defining one component would be to take the difference between any two of the determinations such as $C - A$. The rule for the derivation of the second component is best illustrated

by setting up the first comparison as follows:

A	B	C
-1	0	+1

The numbers are referred to as coefficients and for any such comparison the sum of these coefficients must be zero. The second and independent comparison must be built up so that the sum of the coefficients is zero and also the sum of the products of the coefficients for the two comparisons. The second comparison can now be set up below the first.

A	B	C
-1	0	+1
+1	-2	+1

On examining the coefficients in each line we note that they conform with the rule that their sums and sums of products must be zero.

The next step is to calculate the sums of squares for the two components. The first one is given by $(C - A)^2/2$, and the second by $(A + C - 2B)^2/6$, where the rule for the divisor is to take the sum of the squares of the coefficients. If we add the above two sums of squares we will get the sum of squares for the two degrees of freedom calculated in the ordinary way. This can be used as a check on the calculations.

It should be obvious that the procedure of splitting up the degrees of freedom and the corresponding sums of squares may have a very definite logical basis. This is particularly true if A, B, and C represent three levels of one factor as in an experiment on loaf volume in which the levels are 1, 2, and 3 mg of bromate added to the dough. In such an experiment some sort of trend in the results would be expected, and it would be logical to compare the volume for the smallest quantity of bromate with the volume for the largest amount of bromate added. Actually the method being used here is a short cut to analyses by means of regression and correlation coefficients. The effect measured by $C - A$ is actually the same as that which would be measured by a regression straight line fitted to the three points, in which the level of bromate is the independent variable and loaf volume the dependent variable. In terms of regression analysis the sum of squares calculated as above for $C - A$ is $[\Sigma(xy)]^2/\Sigma(x^2)$, where x and y are measured from their means, x being the independent variable and y the dependent variable. This is the portion of the total sum of squares of y that is accounted for by the regression straight line. The single degree of freedom for $C - A$ represents the linear regression function, and the

single degree of freedom for $A + C - 2B$ represents the additional constant involved in fitting a quadratic function of the form $Y = a + bx + cx^2$.

We should notice in the first place that there are three different ways in which the two degrees of freedom can be split up into linear and quadratic components. This will depend entirely on the choice of the experimenter, but in an example such as the one mentioned above, the linear component will usually be $C - A$. The most logical division, however, will arise from the general characteristics of the experiment. Suppose that A represents a zero level of some treatment and B and C are the two actual levels. One of the most interesting comparisons is therefore $B + C - 2A$, which is equivalent to comparing treatment with no treatment.

If there are more than three levels of the one factor, and these are to be split up into orthogonal degrees of freedom having some logical value for the experiment, we can proceed in a similar manner. For example, with four levels represented by A, B, C , and D , the linear quadratic and cubic components are as follows:

A	B	C	D	
+3	+1	-1	-3	Linear component
-1	+1	+1	-1	Quadratic component
+1	-3	+3	-1	Cubic component

It is only necessary to have the coefficients and any similar problem can be solved. These are given in the tables by Fisher and Yates (1938) for fitting components up to the 5th degree for any number of levels up to 52. The divisors for obtaining the sums of squares are also given in these tables and this saves a good deal of labor when working with five or more levels.

A different method of splitting up the degrees of freedom is indicated in certain examples. Suppose that A and B are two levels of one improver and C and D are two levels of another improver. Here, two comparisons are obvious, and then the third comparison will be fixed. The three comparisons are as follows:

A	B	C	D
-1	-1	+1	+1
-1	+1	-1	+1
-1	+1	+1	-1

The first comparison is between the two kinds of improvers. The

second is between the two levels for both improvers. The third represents the extent to which the improvers give different results at the two levels, and is comparable therefore to an interaction between improvers and levels.

With this brief discussion of methods of splitting up degrees of freedom into logical components, we are ready to consider the analysis of a hypothetical experiment. Let us suppose that five flours are tested with four levels of bromate added; for example, with 1, 2, 3, and 4 mg. The data could be arranged in the form of a table as follows, and for each variety we shall determine the totals, the linear components, the quadratic components, and the cubic components.

Flours	Mg bromate				Variety totals	Linear components	Quadratic components	Cubic components
	1	2	3	4				
A								
B								
C								
D								
E								
Totals								

The analysis of variance can now be outlined. It will be noted that the analysis differs from a simple analysis in that the treatment sum of squares and the interaction sum of squares are broken up into

OUTLINE OF ANALYSIS OF VARIANCE

	DF	Mean square
Varieties	4	v
Treatments—linear effects	1	1
—quadratic effects	1	q
—cubic effects	1	c
Interaction—linear effects \times varieties	4	$1 \times v$
—quadratic effects \times varieties	4	$q \times v$
—cubic effects \times varieties	4	$c \times v$

appropriate linear, quadratic, and cubic components. In a simple analysis, the interaction mean square would be used to test the significance of the mean squares for varieties and treatments. In the above form the mean square for the interaction can likewise be used to test the three components of the treatment effect. The results will show whether or not there is a tendency towards a definite type of trend. It is possible, in addition, to make a test of the linear and quadratic portions of the interaction. This is done by performing two supplementary tests, setting up an analysis for each. These are as follows:

	DF	MS	F
Linear effect \times varieties	4		
$q + c + (q \times v) + (c \times v)$	10		
	DF	MS	F
Quadratic effect \times varieties	4		
$c + (c \times v)$	5		

The general procedure followed in these supplementary analyses is the same as in the ordinary covariance analysis; except that in the first place the procedure here is shown in relation to the sums of squares and degrees of freedom of the ordinary analysis of variance, and in the second place it is carried forward past the fitting of linear regressions to the fitting of second and third degree constants to the results for the treatments. If we were dealing with the linear effects only, we would have divided the treatment degrees of freedom into two portions; linear regression and deviations from linear regression, for 1 and 2 degrees of freedom, respectively. The interaction would have been divided correspondingly and we would have carried out only the first of the supplementary tests mentioned above.

It is important to note the exact relation of the supplementary tests made here to the tests for heterogeneity of regression applied in the covariance analysis. The first test of the interaction of the linear effects with varieties is a test of the heterogeneity of the linear regres-

TABLE III

LOAF VOLUMES FOR 17 VARIETIES OF WHEAT TESTED AT FOUR LEVELS OF POTASSIUM BROMATE ADDED IN THE BAKING PROCEDURE

Variety	Levels of bromate in mg				Total	Lin. comp.	Quad. comp.	Cu. comp.
	1	2	3	4				
1	10.8	10.6	9.8	8.8	40.0	-6.8	-0.8	0.4
2	9.8	9.6	8.6	8.2	36.2	-5.8	-0.2	1.4
3	8.5	8.2	7.7	7.4	31.8	-3.8	0	0.4
4	8.2	7.6	7.2	7.0	30.0	-4.0	0.4	0
5	10.4	10.6	9.8	9.4	40.2	-3.8	-0.6	1.4
6	9.6	9.8	9.2	8.4	37.0	-4.2	-1.0	0.6
7	9.0	9.0	8.8	7.8	34.6	-3.8	-1.0	-0.6
8	8.6	8.7	8.5	8.5	34.3	-0.5	-0.1	0.5
9	9.4	10.0	9.6	9.6	38.6	0.2	-0.6	1.4
10	10.0	10.2	9.6	9.0	38.8	-3.6	-0.8	0.8
11	9.4	9.6	9.5	9.2	37.7	-0.7	-0.5	0.1
12	8.4	8.7	8.8	8.8	34.7	1.3	-0.3	0.1
13	6.6	6.5	6.8	6.6	26.5	0.3	-0.1	-0.9
14	9.1	8.9	8.4	7.8	34.2	-4.4	-0.4	0.2
15	10.8	10.7	10.2	10.0	41.7	-2.9	-0.1	0.7
16	7.5	7.4	7.2	7.2	29.3	-1.1	0.1	0.3
17	8.4	8.6	8.2	8.2	33.4	-1.0	-0.2	1.0
Totals	154.5	154.7	147.9	141.9	599.0	-44.6	-6.2	7.8

sion coefficients. Then, by a simple extension of the procedure, we test the heterogeneity of the quadratic regressions. This process can be carried forward indefinitely. If the number of levels is greater than four, we may, for example, wish to test the heterogeneity of the cubic regressions. With a still greater number of levels it would be possible to make further tests, but it is not often that we will be interested in effects higher than those of the third degree.

We shall now apply the above method to a set of data obtained by Larmour (1941) on the reactions of a series of flours from 17 varieties that were baked with four levels of potassium bromate. These data are given in Table III together with a portion of the calculations. In the actual experiment another treatment was included in which no bromate was added. This level is omitted here in order to simplify the example. The loaf volumes, which were given by Larmour in cc, have here been coded by dividing by 100 and rounding off the last decimal figure. Linear, quadratic, and cubic components have been calculated for each variety and are given in the last three columns of the table.

In the first place, the usual analysis is set up giving the sums of squares of varieties, treatments, and interaction. This works out as follows:

	Degrees of freedom	Sums of squares	Mean square	F	5% point
Varieties	16	70.0097	4.376	40.1	1.86
Treatments	3	6.5947	2.198	20.2	2.80
Interaction	48	5.2303	0.1090		

The next step is to split up the treatment sum of squares into linear, quadratic, and cubic components. These are calculated from the treatment totals of Table III.

Linear component

$$= \frac{(-3 \times 154.5 - 1 \times 154.7 + 1 \times 147.9 + 3 \times 141.9)^2}{20 \times 17} = 5.8505$$

Quadratic component

$$= \frac{(1 \times 154.5 - 1 \times 154.7 - 1 \times 147.9 + 1 \times 141.9)^2}{4 \times 17} = 0.5653$$

Cubic component

$$= \frac{(-1 \times 154.5 + 3 \times 154.7 - 3 \times 147.9 + 1 \times 141.9)^2}{20 \times 17} = 0.1789$$

The coefficients used in the above equations can be obtained from the tables by Fisher and Yates (1938).

To determine the corresponding interactions we deal with the figures in the three columns on the right of Table III. Thus:

Linear effects \times varieties

$$= [(6.8^2 + 5.8^2 + \dots + 1.0^2)/20] - 5.8505 = 4.2665$$

Quadratic effects \times varieties

$$= [(0.8^2 + 0.2^2 + \dots + 0.2^2)/4] - 0.5653 = 0.6297$$

Cubic effects \times varieties

$$= [(0.4^2 + 1.4^2 + \dots + 1.0^2)/20] - 0.1789 = 0.3341$$

Note that the correction terms are the components previously calculated in dividing up the sums of squares for treatments. The complete analysis can now be set up. The tests of significance emphasize that

	DF	SS	MS	F	5% point of F
Linear effect	1	5.8505	5.8505	53.7	4.04
Quadratic effect	1	0.5653	0.5653	5.19	4.04
Cubic effect	1	0.1789	0.1789	1.64	4.04
Interaction	48	5.2303	0.1090		

the large differences between the treatments are mainly linear effects; in other words, there is a definite tendency, when all varieties are combined, for the quantities of bromate added to bring about a proportionate reduction in the loaf volumes. However, the quadratic effect is also significant; and on examining the totals for the treatments, we note that there is a slight increase in volume from level one to level two and a definite falling off of the volumes for the third and fourth levels. The cubic effect is insignificant, and this is exactly what we would expect from an examination of the treatment totals.

In view of the fact that the linear and quadratic effects are significant, it is of interest to examine the components of the interaction. We can make two simple tests as follows:

	DF	SS	MS	F	5% point of F
Lin. \times varieties	16	4.2665	0.2666	5.31	1.95
Error	34	1.7080	0.05024		
Quad. \times varieties	16	0.6297	0.03936	1.30	2.29
Error	17	0.5130	0.03018		

In order to obtain the error for testing the linear interaction effect, we use all the sums of squares representing deviations from linear regression. We have, therefore, $0.5653 + 0.1789 + 0.6297 + 0.3341 = 1.7080$. Similarly the error for testing the quadratic interaction effect contains all the sums of squares representing deviations from quadratic regression. This is $0.1789 + 0.3341 = 0.5130$.

These tests have furnished information of value. They have shown that, although the linear effect for all varieties combined is significant, the linear effects for the varieties taken individually are not uniform. It is not possible, however, to detect any differences between the quadratic effects.

We can again show that what we have done in the last two tests is exactly the same as is done in the covariance analysis when testing the heterogeneity of regressions. The method for setting up the analysis of covariance is as follows:

Total SS	DF	Linear regression SS	DF	Residual	DF	Mean square	F	5% point
11.8250	51	10.1170	17	1.7080	34			
11.8250	51	5.8505	1	5.9745	50			
Error Heterogeneity of regressions				1.7080 4.2665	34 16	0.05024 0.2666	5.31	1.95

In a similar manner we can set up the covariance analysis for the quadratic effects.

Total SS	DF	Quadratic regression SS	DF	Residual	DF	Mean square	F	5% point
1.7080	34	1.1950	17	0.5130	17			
1.7080	34	0.5653	1	1.1427	33			
Error Heterogeneity quad. regression				0.5130 0.6297	17 16	0.03018 0.03936	1.30	2.29

Selection of Levels

If it is agreed that the analytical methods demonstrated in the last example are useful and practical, a further point can be made in connection with experimental design. Since the simplicity of the method arises chiefly from the fact that the levels of the factor being studied are in arithmetical progression, it is suggested that time and effort can be saved by careful attention to the levels used. It happens frequently that the experiment calls for the use of levels that are not in arithmetical progression, and the experimenter may inadvertently use a series of levels that are not in any sort of mathematical progression. This is very likely to make the calculations in any method of analysis much more difficult, and it is particularly true with the method of analysis described above.

Let us suppose for example that the dependent variable being studied is expected to react as indicated by the following hypothetical data.

Levels (x)	0.10	0.25	1.00	5.00	20.00
Determinations (y)	15.0	15.3	15.7	15.6	16.0

The levels here are of the kind that are sometimes arbitrarily selected without giving much thought to methods of analysis. At low levels of x , much smaller increases in x are required to produce a given increase in y than at higher levels of x . The obvious procedure in this example is to set up levels such that their logarithms are in arithmetical progression. We could use the levels 0.10, 0.40, 1.6, 6.4, 25.0. The hypothesis that there is a linear relationship between values of y and the logarithms of x could then be tested in a very simple manner by replacing the values of x by 1, 2, 3, 4, 5. Most experiments of this type are for the purpose of testing a hypothesis of this kind. There are a great variety of hypotheses that can be tested, but, generally speaking, these can be expressed as some form of mathematical relation, and the levels set up can be such that they may be transformed readily to a series of natural numbers. Actually there is no excuse for a series of levels that do not form a regular series of some sort. With this in mind it should be possible to make the statistical work on many problems much easier than would otherwise be the case.

Summary

There is a very close relation between methods of analysis and experimental design. A study of different methods of analysis may suggest new designs or changes in others.

A description of a simple covariance analysis is given, with the object of demonstrating how the method can be used to advantage in certain types of experiments.

The procedure of splitting up degrees of freedom in the analysis of variance is described, and an example given showing how the maximum amount of information can be extracted from an experiment in which one of the factors is tested at various levels.

Reference is made to the possibility of simplifying calculations by being careful in the selection of levels in a factorial experiment.

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EFFECT OF ENVIRONMENT DURING THE GROWTH AND DEVELOPMENT OF WHEAT ON THE BAKING PROPERTIES OF ITS FLOUR¹

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Recent investigations have shown that there is a linear relation between protein content and loaf volume *within* wheat varieties and a variation in baking quality *between* varieties (Aitken and Geddes, 1934 and 1939; Larmour, 1931; Larmour, Working, and Ofelt, 1939 and 1940; McCalla, 1940; Bayfield, Working, and Harris, 1941; and Johnson, Swanson, and Bayfield, 1943).³ The exceedingly high correlations between protein and volume obtained in the more recent work indicate that within varieties there was little variation in quality, practically the entire variation being in the amount of protein. Accordingly, since differences in protein content are due largely to differences in the environments under which the wheats are grown (McCalla and Rose, 1941), one obtains the impression that environment has little effect on quality. However, it should be noted that much of this work was done on composite samples, the composites representing a number of localities which produced similar protein content, or the results were reported as the averages of loaf volumes for each increment of protein content. Marked environmental effects occurring in a few localities or small effects in a number of localities might be largely eliminated by these procedures.

Sandstedt and Ofelt (1940), by diluting flours to a given protein content with starch, showed that within a variety the baking quality seemed to decrease with an increase in protein content. However, they pointed out that this was not true for all varieties and that high-protein samples grown in one particular locality had outstanding quality. This suggested that the quality of these high-protein flours was due to the environment under which they were grown.

The purposes of the present paper are to offer further evidence of the considerable differences in baking properties which may be obtained by growing wheat in different localities and to show that the baking quality of flour may be materially dependent on the conditions under which the wheat is grown.

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³ Some seeming contradiction arises owing to the quite common use of *protein content* as a measure of *quality* in *wheat*. For example, McCalla and Rose (1941) concluded that environment was more effective in determining *quality* of *wheat* than was variety.

Materials and Methods

Wheat Origin and Characteristics. The Agronomy Department of the Nebraska Experiment Station, the Agricultural Extension Service, and the Nebraska Grain Improvement Association each year conduct regional performance tests of promising new strains in comparison with standard varieties. These tests are so planned that all varieties grown in one locality are comparable. In the fall of 1939, 22 such tests were planted. Because of an exceedingly dry fall in the south central part of the state with the consequent poor germination and severe winter-killing, only 14 of these were harvested, as shown in Figure 1. The

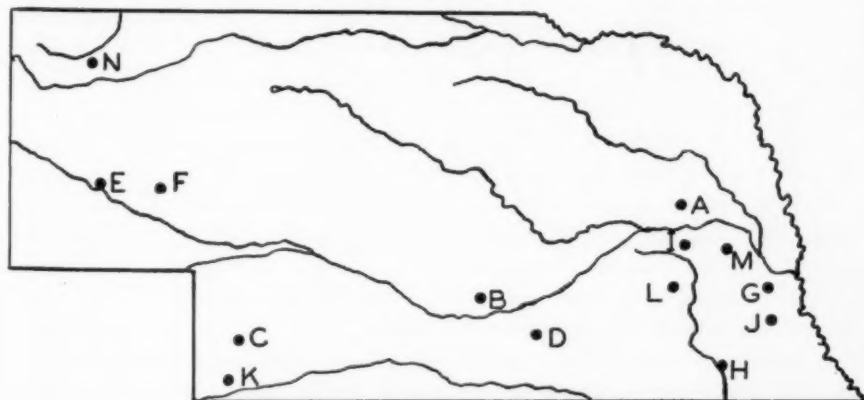


Fig. 1. Location of cooperative wheat test plots—1940.

grain from six varieties included uniformly in all of the tests provides the basis for the studies herein reported.

In order to show the relation of protein to loaf volume to the best advantage, the test localities are designated by letters; the locality which produced the lowest average protein content is designated as *A*, that producing the next higher average protein as *B*, etc. These locality designations apply to all varieties regardless of their individual protein content.

The yields and test weights (Table I) of the wheats and the protein content of the flours from the wheats (Table II) are presented as indications of the wide variation in the environmental conditions prevailing during the 1939-1940 growing season, causing a range in average yield from 13.6 bu at *N* to 38.3 bu at *B*, and from 12.4% protein in the flour at *A* to 16.9% at *N*. However, such wide variations may be expected in the Great Plains region. Nebraska, itself, has a considerable range both in soil type and in climate. The test weights may be considered as indicative of the condition of the grain and of its approximate market grade.

TABLE I

COMPARATIVE YIELDS AND TEST WEIGHTS OF WINTER WHEAT VARIETIES IN COOPERATIVE VARIETY DEMONSTRATION PLOTS, 1940¹

Locality	Mean of all varieties		Variety	Mean of all localities	
	Yield	Test weight		Yield	Test weight
	<i>bu/acre</i>	<i>lb/bu</i>		<i>bu/acre</i>	<i>lb/bu</i>
A	34.3	60.1	Turkey	22.1	58.3
B	38.3	61.5	Nebred	23.3	59.5
C	34.4	61.0	Tenmarq	24.4	57.5
D	21.6	54.4	Cheyenne	24.4	59.4
E	23.7	59.0	Blackhull	24.9	59.8
F	16.3	58.1	Chiefkan	26.0	60.0
G	22.0	59.2			
H	29.4	59.9			
I	26.9	59.8			
J	23.0	60.2			
K	18.2	59.6			
L	17.4	59.9			
M	19.0	59.8			
N	13.6	55.0			

¹ "Results of the 1940 cooperative small grain variety demonstration trials," G. T. Webster, D. L. Gross, and T. A. Klesselbach.

Milling. The wheats were milled to 85% patent flours on a four-stand Allis-Chalmers experimental mill.

Baking Method. The micro-baking technique described by Van Scoyk (1939) as modified by Sandstedt and Ofelt (1940) was used. The baking formula was as follows: 25 g of flour (15% moisture basis), 3% yeast, 6% sugar, 1% salt, 3% shortening, 0.2% malt flour, and 0.001% potassium bromate. Each dough was given *optimum* mixing in a National micromixer. The optimum, which is essentially *development to optimum consistency*, was determined by observing the transition of the dough during mixing from the rough appearance of an undermixed dough to the "velvety smoothness" of a well-developed dough. Proofing was to 6.5 cm height in the tall form micro pans. Flours of the same variety from all localities were baked on one day. This bake was repeated on another day; accordingly, each flour was baked in duplicate, the duplicates being baked on different days. Using this order of baking, differences in results owing to locality become highly significant, especially if the locality effect tends to be similar for all varieties.

The precision which may be attained by an experienced baker when using this experimental baking procedure is shown by the analysis of variance given in Tables III, IV, V, and VI. For testing the variability resulting from the baking procedure itself, the mean square of the remainder was used as error. Calculated on this basis the error of

the difference between two flours (each baked in duplicate) was 6.5 sec in mixing time and 3.2 cc in volume. Accordingly, the differences required for significance (based on the 5% point) would be 20 sec and 10 cc.

Sandstedt and Ofelt (1940) proposed that experimental flours of varying protein content be diluted to a common protein basis for quality determination. Accordingly, each flour was diluted with wheat starch to an artificial 10% protein content and again baked in duplicate, using the same formula and baking procedure as used for the original flours. The starch used for dilution was prepared in the laboratory, discarding the "amylodextrin" fraction (Sandstedt, Jolitz, and Blish, 1939).

Analysis of Variance. The analysis of variance was made according to procedures outlined by Snedecor (1937). All calculations were based on the original determinations (mixing times and loaf volumes), not on the averages of duplicates as given in the tables. As already stated, the mean square of the remainder was used as the error for calculating the standard deviation and error of difference between two flours in showing the precision of the baking procedure. It was also used for testing the significance of the variety \times locality interaction. However, as it was desired to test for significant differences between localities considering all varieties, the mean square of the interaction $V \times L$ must be used as error as this value is shown to be significant. Accordingly, as given in the tables, the error of the difference between two flours and the difference required for significance of the varietal and locality means are calculated on this basis.

Definition of Quality. Larmour, Working, and Ofelt (1939) defined baking quality as the capacity of a flour to fulfill the loaf volume prediction made on the basis of its protein content. Quality when used in this paper is limited to the above definition.

Experimental Results and Discussion

Flour Protein. As previously stated, the range in protein content of the flours (Table II) indicates a wide variation between the environments under which the wheats were grown. The Turkey and Black-hull wheats were slightly higher in protein than the other varieties, but there was no consistent tendency for one variety to produce significantly more or less protein than another. The differences between varieties were slight compared to the differences between localities. Similar results were reported by McCalla and Rose (1941) and by a number of earlier investigators. Bailey (1925) gives a comprehensive review of the extensive literature on the influence of environment on the *composition* of wheat.

TABLE II
EFFECT OF ENVIRONMENT AND VARIETY ON FLOUR PROTEIN CONTENT¹

Locality	Flour protein content (15% moisture basis)						
	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	%	%	%	%	%	%	%
A	11.6	12.5	12.2	12.5	12.7	12.8	12.4
B	13.2	12.5	11.4	12.4	13.2	12.2	12.5
C	13.1	12.5	12.8	13.1	13.6	13.2	13.0
D	14.3	13.1	13.6	13.3	13.7	13.1	13.5
E	14.6	14.1	14.2	13.8	14.4	13.9	14.2
F	15.3	15.0	14.7	14.8	14.7	14.6	14.8
G	15.8	14.9	14.8	13.9	16.4	15.1	15.1
H	15.2	15.0	15.9	15.2	15.4	14.4	15.2
I	16.0	15.3	15.3	15.4	15.3	14.7	15.3
J	15.4	15.8	15.6	15.2	15.3	15.4	15.4
K	15.9	15.5	15.8	15.6	15.5	15.0	15.5
L	15.7	15.4	15.7	15.2	15.8	15.3	15.5
M	16.9	16.9	16.3	15.3	15.8	16.0	16.2
N	17.4	17.5	16.7	16.7	16.5	16.5	16.9
Mean	15.0	14.7	14.6	14.4	14.9	14.4	

¹ Cooperative wheat tests (1940).

Absorption. In baking these samples it soon became apparent that there were a number of flour characteristics which varied with changes in environment and that in most cases the varieties tended to respond in a similar manner to any particular change in environment, e.g., all flours from locality *N* had unusually high absorptions; 2 to 4% higher than when grown in other localities which produced a similar quantity of protein. Locality *N* produced the highest protein wheats, but the difference in protein quantity between localities *M* and *N* was not great enough to account for the difference in absorption.

Handling Properties. The handling properties of the doughs from wheats grown at *A* varied considerably from the handling properties of the same varieties grown in the other localities. The flours from this locality had a tendency to produce soft and somewhat sticky doughs. This was particularly evident when comparing localities *A* and *B*, which produced flours with similar protein content.

Mixing Requirements. The mixing requirements of the doughs, the time required to reach *optimum development*, varied markedly with environment. This is shown by the data and analysis of variance presented in Tables III and IV. The mixing requirements are given as the time in minutes required to reach optimum consistency; each figure being the average of duplicate mixes. The average mixing time for the series of flours with natural protein content was 2 min 17 sec; that for those diluted to 10% protein content was 2 min 23 sec.

TABLE III
EFFECTS OF ENVIRONMENT AND VARIETY ON MIXING REQUIREMENT OF FLOURS
(Optimum mixing time—average of duplicates)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>
A	2.45	2.38	2.09	3.96	1.58	1.54	2.33
B	1.92	2.92	2.38	2.92	1.50	1.54	2.19
C	1.79	2.13	1.71	3.54	1.38	1.38	1.99
D	2.67	4.42	2.71	4.75	1.83	1.71	3.01
E	1.88	2.79	1.96	3.13	1.67	1.50	2.15
F	2.00	3.13	1.96	3.79	1.58	1.54	2.33
G	1.75	2.84	2.30	4.05	1.50	1.67	2.35
H	1.79	2.75	2.04	3.63	1.54	1.54	2.21
I	1.63	2.38	1.88	2.79	1.46	1.38	1.92
J	1.67	2.46	2.00	3.42	1.46	1.50	2.08
K	1.63	1.92	1.48	2.46	1.42	1.33	1.71
L	1.88	2.92	2.25	3.92	1.67	1.42	2.34
M	1.75	2.58	2.04	2.88	1.46	1.42	2.02
N	2.67	4.17	3.25	5.13	2.42	1.80	3.24
Mean	1.96	2.84	2.15	3.60	1.61	1.51	
Correlation coefficient, protein \times mixing time	0.20	0.12	0.08	0.04	0.24	0.10	

Difference Required for Significance of Protein-Mix time: $r = 0.53$

ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	17.95	95.0**	Error of difference between two
Locality	13	1.98	10.5**	flours = 0.309
Interaction	65	0.189 ¹	7.98**	Based on the 5% point, difference
V \times L				required for significance of:
Remainder	84	0.0237		Varietal means = 0.233
Total	167			Locality means = 0.355

¹ Used as error.

** Significant at 1% point.

The significance of differences in mixing time is indicated by the analysis of variance. Differences between locality averages greater than 21 sec, and between variety averages greater than 14 sec (differences required for significance based on the 5% point) may be considered as significant.

All varieties grown at locality *K* required a short mixing time, while those grown at *N* required a long mixing. Chiefkan (noted for its short mixing requirement) from *N* required as much mixing as Turkey or Tenmarq from *K*. Blackhull grown at *N* required more mixing than these other varieties grown at *K* and even as much as Cheyenne (normally requiring an exceedingly long mix) grown at *K*. It is interesting, though perhaps to be expected, that the difference between

TABLE IV
EFFECT OF ENVIRONMENT AND VARIETY ON MIXING REQUIREMENTS OF FLOURS
(Diluted to 10% protein content)
(Optimum mixing time—average of duplicates)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>
A	2.46	2.17	2.08	3.92	1.71	1.54	2.31
B	1.79	3.13	2.46	3.00	1.54	1.59	2.25
C	1.80	2.25	2.00	3.54	1.46	1.50	2.09
D	2.46	3.95	2.71	5.42	1.83	1.79	3.03
E	1.79	2.88	2.00	3.59	1.75	1.50	2.24
F	1.88	3.13	2.25	4.29	1.63	1.71	2.48
G	1.75	2.88	2.46	4.71	1.50	1.75	2.51
H	1.63	2.79	2.33	3.67	1.50	1.67	2.26
I	1.72	2.42	1.92	3.21	1.46	1.46	2.02
J	1.58	2.58	2.17	3.92	1.46	1.58	2.22
K	1.71	2.00	1.58	2.63	1.38	1.42	1.78
L	1.75	2.96	2.25	4.21	1.80	1.75	2.45
M	1.75	2.84	2.25	2.92	1.75	1.46	2.16
N	2.58	4.50	3.59	5.84	2.42	1.96	3.48
Mean	1.89	2.89	2.29	3.92	1.66	1.62	

ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	22.25	86.6**	Error of difference between two
Locality	13	2.18	8.48**	flours = 0.359
Interaction	65	0.257 ¹	26.5**	Based on the 5% point, difference
V × L				required for significance of:
Remainder	84	0.00969		Varietal means = 0.272
Total	167			Locality means = 0.414

¹ Used as error.

** Significant at 1% point.

varieties is less in the localities requiring short mixing times and greater in localities requiring long mixing times; the difference between Chiefkan and Cheyenne from K was only 1.1 min, while at N the difference was 3.4 min. Also, the difference between localities was less for varieties having short mixing requirements and greater for varieties having long mixing requirements; the difference between Chiefkan wheats from K and N was 0.5 min, while for Cheyenne it was 2.7 min.

Relation of Protein Content to Mixing Time. The mixing requirements of the diluted flours (Table IV) are fairly good checks of the mixing requirements of the corresponding flours of natural protein content. This is strong evidence that protein content does not affect the mixing requirement as determined by this method. Further substantiation is obtained from the correlation between the mixing requirement and protein content for each variety. These correlation

coefficients ranging from .04 to .24 are given in Table III. They are not significant. It is quite evident that the protein content had little if any effect on the mixing requirement. These data substantiate conclusions drawn from mixogram studies on flours, and on flours diluted with starch to various protein levels, by Ofelt and Sandstedt (1941) and by Johnson, Swanson, and Bayfield (1943).

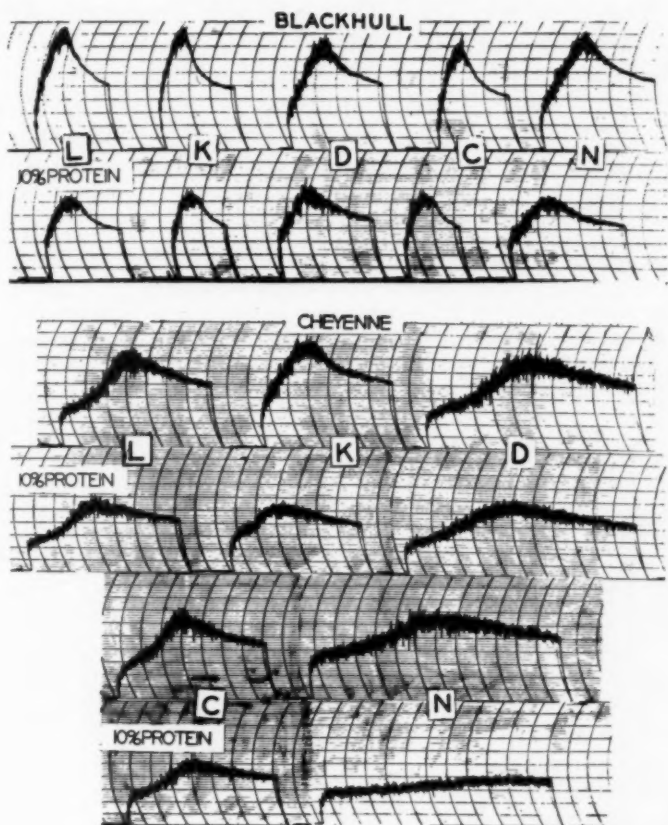


Fig. 2. Effect of environment on type of mixograms produced by Blackhull and Cheyenne wheat flours. Upper curves were made with flours of natural protein content; lower curves with the flours diluted to 10% protein level.

Mixograms. The differences in mixing characteristics of flours from various localities may be shown quite effectively by means of mixograms (recording dough mixer curves). All mixograms of this series of flours were made using the *baking absorptions*, i.e., the absorptions determined for use in the baking procedure. Cheyenne and Blackhull are representative of varieties requiring exceedingly long and short mixing. The mixograms for these varieties grown in environments L, K, D, C, and N (Figure 2) are used to illustrate the differences in

mixing characteristics which may be produced by environment in flours of the same variety with nearly equal protein content, the pairs *L K* and *D C* being comparable (Table II). The upper curves were obtained from the flours with their natural protein content, while the lower curves were obtained from the flours diluted to 10% protein with wheat starch. Dilution to a 10% protein content for determining curve characteristics makes all curves directly comparable (Ofelt and Sandstedt, 1941).

While in general the curves for the two varieties were typical, the environment produced, in each variety, a wide range in curve character with the curve for Blackhull grown at *N* tending to approach in character the curve for Cheyenne grown at *K*. Swanson (1939) showed

TABLE V
EFFECT OF ENVIRONMENT AND VARIETY ON LOAF VOLUME
(Loaf volume—average of duplicates)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	cc	cc	cc	cc	cc	cc	cc
A	161	164	170	168	169	154	164
B	158	160	160	156	155	138	155
C	165	164	169	165	162	145	162
D	207	209	203	189	184	154	191
E	163	170	172	168	159	145	163
F	166	184	186	168	162	142	168
G	182	204	197	189	175	159	184
H	180	206	191	181	170	154	180
I	186	217	199	190	175	157	187
J	199	219	208	201	185	163	196
K	173	193	175	167	164	142	169
L	200	218	219	185	175	153	192
M	201	229	218	197	169	155	195
N	188	204	212	171	185	152	184
Mean	181	196	192	178	171	151	
Regression coefficient, loaf volume on protein content	6.18	11.0	9.10	4.13	4.0	3.1	

ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	7233	48.7**	Error of difference between two flours = 8.6 cc
Locality	13	2357	15.9**	Based on the 5% point, difference required for significance of:
Interaction V × L	65	148 ¹	7**	Varietal means = 6.5 cc
Remainder	84	20.0		Locality means = 9.9 cc
Total	167			

¹ Used as error.

** Significant at the 1% point.

TABLE VI
EFFECT OF ENVIRONMENT AND VARIETY ON LOAF VOLUME OF FLOURS
(Diluted to 10% protein content)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	cc	cc	cc	cc	cc	cc	cc
A	159	142	143	144	140	138	144
B	147	143	142	142	130	124	138
C	152	141	139	137	137	128	139
D	162	164	153	154	141	138	152
E	142	137	137	137	125	126	134
F	139	138	137	132	128	120	132
G	148	147	143	140	129	129	139
H	144	146	139	133	131	125	136
I	145	145	141	135	132	123	137
J	149	145	144	136	136	128	140
K	135	134	129	117	118	115	125
L	145	149	144	133	127	126	137
M	142	145	142	132	127	119	135
N	139	134	141	119	131	121	131
Mean	146	144	141	135	131	126	
Protein-volume regression coefficient	-3.18	-1.30	-0.70	-5.5	-2.26	-2.76	

ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	1769	68**	Error of difference between two
Locality	13	485	19**	flours = 3.6 cc
Interaction	65	26.1 ¹	5.3**	Based on the 5% point, difference
V × L				required for significance of:
Remainder	84	4.88		Varietal means = 2.7 cc
Total	167			Locality means = 4.2 cc

¹ Used as error.

** Significant at the 1% point.

similar wide variations in mixograms obtained from flours produced under different environments.

Statistical Significance of Loaf Volume Data. The loaf volume data obtained from baking this series of flours at the natural protein level together with an analysis of variance of the data are given in Table V and for the diluted flour samples in Table VI. The analysis of variance shows that both the variety and the environment were responsible for highly significant variations in loaf volume, not only in the flours at their natural protein content but also in the flours diluted to 10% protein content. Differences required for significance were less for the diluted flour data than for the original flour data; this is to be expected since the loaves were much smaller.

Differences between locality means greater than 9.9 cc, or between variety means greater than 6.5 cc (significant differences based on the 5% point), may be considered as significant.

Loaf Volume-Protein Relationships. The relation of flour protein to loaf volume is shown by the regression curves on the left in Figures

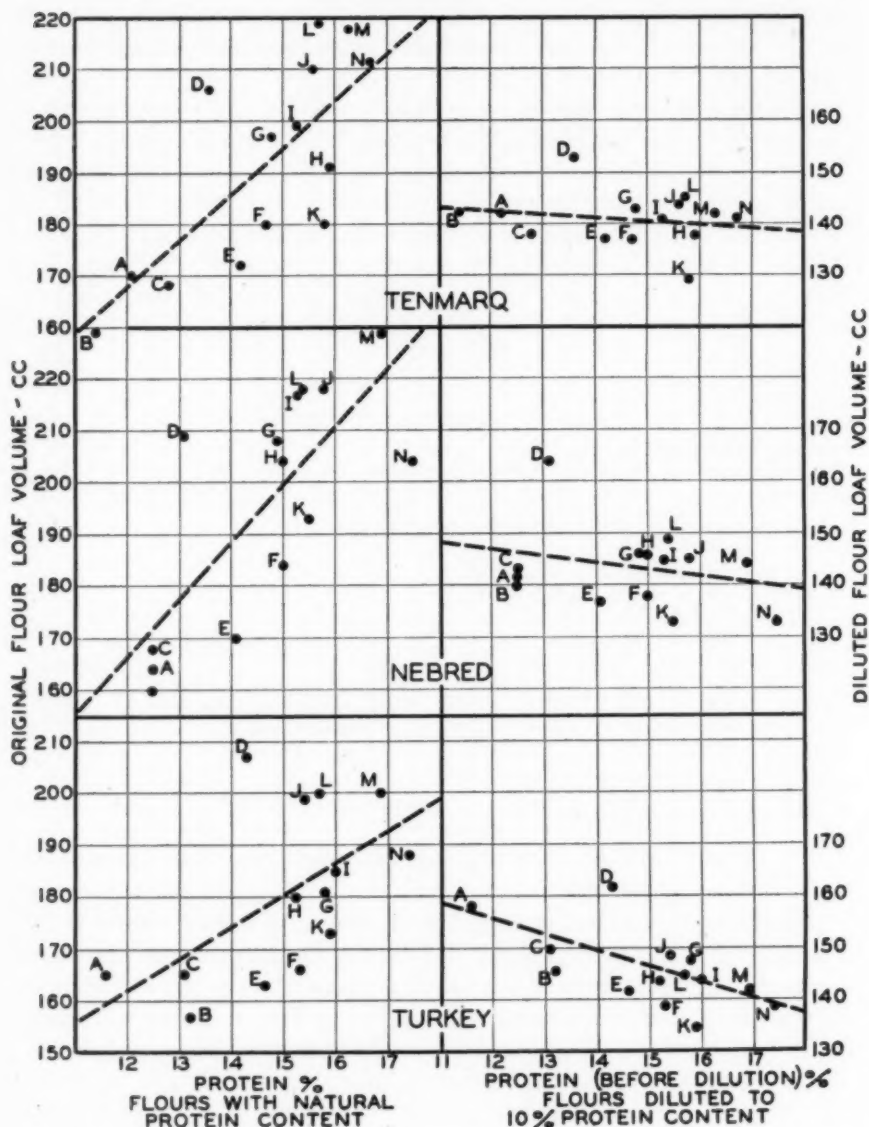


Fig. 3. Effect of variety and of environment on the relationship of loaf volume to protein. Left hand curves are for natural flours; right hand curves are for flours diluted to 10% protein content.

3 and 4. Each curve represents the results obtained from a single variety; the localities are indicated by the letters A, B, etc.

The outstanding characteristic of these curves is the deviation of

the individual samples from the calculated regression line. This is contrary to the marked lack of deviation shown by composited wheat samples (Larmour, Working, Ofelt, 1939, 1940, and McCalla, 1940). It can be seen from inspection of the curves in Figures 3 and 4 that compositing samples of nearly the same protein content or averaging the loaf volumes obtained from those having nearly equal protein (Larmour, 1931) would quite likely eliminate the variations.

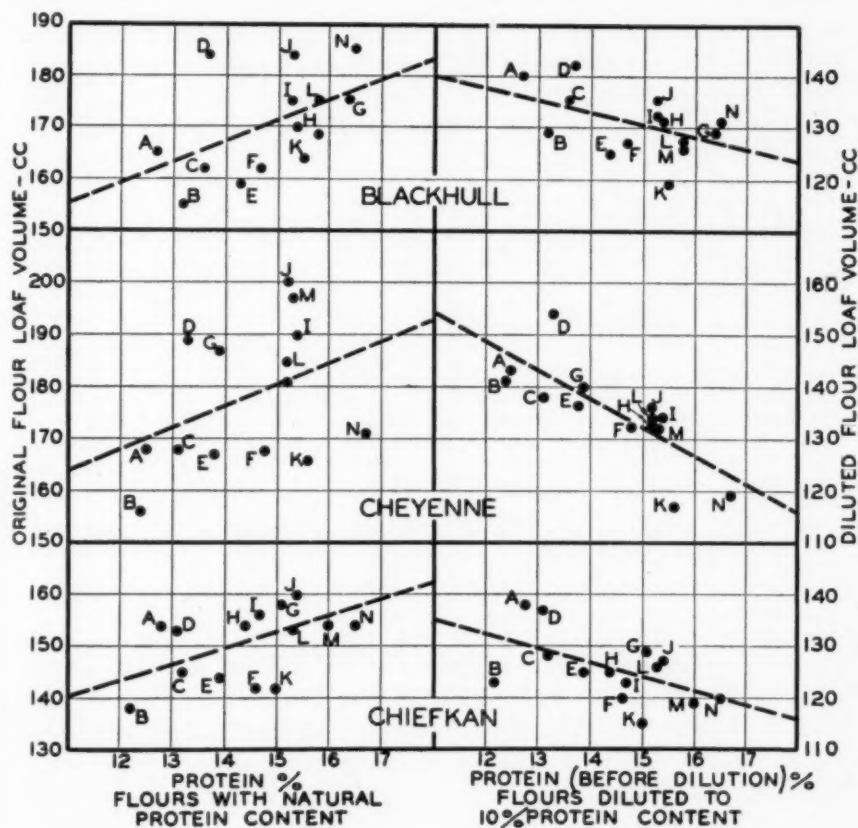


Fig. 4. Effect of variety and of environment on the relationship of loaf volume to protein. Left hand curves are for natural flours; right hand curves are for flours diluted to 10% protein content.

The marked individual variations in loaf volume were not related to protein content; *e.g.*, the environment *D* yielded flour with the capacity to produce exceptionally large loaves, whereas the environments *C* and *E* yielded flours with similar protein content but which were incapable of producing loaves with as great volume. Similarly, the environments *K* and *L* yielded wheats differing little in protein quantity but differing greatly in loaf volume capacity. Locality *D*

produced wheat with greater volume potentiality than locality *K*, even though the flours had nearly a 2% lower protein content. The quality differences were not as pronounced with Chiefkan and Blackhull wheats; nevertheless, the differences are significant and tend to parallel the other varieties at the corresponding localities.

Effect of Varying the Baking Formula. Such wide deviations from the expected volume-protein relationship suggest the possibility that the baking formula was not adapted to the flours from such localities as *C*, *E*, *K*, and *N*, while it might have been optimum for the flours from *D* and *L*. Accordingly, the flours from localities *C*, *D*, *L*, and *K* were rebaked, using 0.002% KBrO_3 instead of 0.001% and again rebaked using 6% dry milk solids and 0.004% KBrO_3 (Ofelt and Larmour, 1940).

TABLE VII
COMPARISON OF LOAF VOLUMES OBTAINED WITH THREE BAKING FORMULAS

Variety	Locality	Flour protein	Loaf volume		
			KBrO_3 1 mg %	KBrO_3 2 mg %	6% D.M.S. +4 mg % KBrO_3
Nebred	N	% 15.4	cc 218	cc 213	cc 215
	F	15.5	193	187	184
	O	13.1	209	203	212
	C	12.5	168	164	163
Tenmarq	N	15.7	219	213	210
	F	15.8	180	165	169
	O	13.6	206	200	206
	C	12.5	169	172	160

The loaf volumes obtained from the Nebred and Tenmarq flours using the three baking formulas are given in Table VII. It is seen that these changes in the baking formula did not materially alter the relative volumes obtained (in fact the volumes obtained with each flour by the three formulas are in surprisingly close agreement). As possible explanations for the lack of variation between these widely differing formulas, it should be noted that absorption and mixing in all cases was "optimum" and that the "rate of proof" was largely eliminated as a factor since proofing was to height. This effect of proofing to height was shown by Sandstedt and Blish (1939).

Loaf Volume-Locality Relationships. A comparison of the protein-volume curve for one variety with the curves of the other varieties (Figures 3 and 4) shows that, with few exceptions, the six varieties tended to respond in a similar manner to any particular set of environmental conditions; *i.e.*, flours from certain localities are found above

the regression line regardless of variety, and flours from certain other localities are found below the regression line. However, the differences between localities are greater for certain varieties than for others. In order to show this tendency to better advantage, the Turkey wheat flour samples were arbitrarily arranged in the order of increasing loaf volumes without regard to protein content. The loaf volumes in this order were then plotted against locality; thus a fairly smooth curve for the Turkey wheat flours was obtained (Figure 5). The loaf volumes

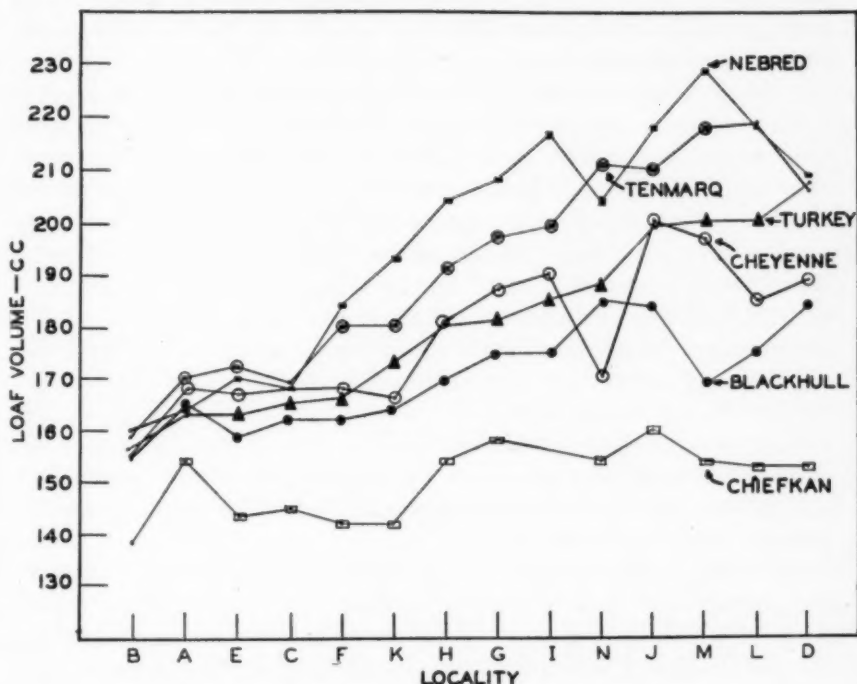


Fig. 5. Effect of locality and variety on loaf volume at the natural protein levels.

obtained from the other varieties for the respective localities were then introduced on the graph and the curves drawn for each variety. This gives a direct comparison of the loaf volumes obtained from all varieties from each locality.

This evidence gives the same general impression as was obtained from a comparison of the individual curves: the loaf volumes of the varieties had a tendency to vary in the same direction with a change in environment. The most conspicuous exceptions were the Nebred and Cheyenne samples grown under environment *N*.

It is quite evident that in the lower loaf volume ranges, the wheats, excepting Chiefkan, gave similar loaf volumes when grown under the

same environmental conditions, but in the higher loaf volume ranges there was a considerable spread between the varieties. The curve for Chiefkan has a tendency to remain level; *i.e.*, Chiefkan did not respond as readily as the other varieties to changes in environment. Blackhull takes a somewhat intermediate position. On the whole these data indicate that the varieties tend to respond in a similar manner to changes in environment; however, they differ markedly in the amount of response, the response being roughly proportional to the loaf volume potentiality of the variety. The interaction *variety* \times *locality*, being highly significant, also shows that the varieties did not all respond alike to the differences in environment.

Baking Tests with Flours Diluted to 10% Protein. The right hand curves in Figure 3 represent the loaf volumes obtained from the same flours as shown in the left hand curves after they had been diluted with starch to an artificial 10% protein content. This dilution was used to eliminate quantity of protein as a variable not only in estimating quality from loaf volume but also as a variable in evaluating such other characteristics as grain, texture, and handling properties. It is exceedingly difficult to evaluate these factors in flours of unequal protein content since "buckiness" and a tendency toward extreme open grain are characteristic of high-protein strong flours, especially when supplements are used in the baking test to obtain maximum volumes. The 10% protein level was chosen so that the lower protein wheats could be included in this comparison. Generally, there might be considerable advantage in diluting to a protein level similar to that used in average commercial baking.

The loaf volume curves from the diluted flours indicate much the same variation in quality in any one wheat variety as was shown by the data for the original flours. There is an evident similarity between the distribution of the natural and the diluted flours in respect to their regression lines. A comparison of the relation of the individual samples of natural and diluted flours to their respective regression lines indicates that much the same information may be obtained about the individual samples by either baking procedure; the exceedingly high quality of the wheats grown under environment *D* and the poor quality of those grown under environment *K* is apparent.

Relation of Quality to Protein Content. The 14 samples of flour representing each variety are too few to give a dependable varietal regression line; nevertheless, these regression lines for the diluted flours seem to indicate that in the varieties having the highest loaf volume potentialities (Nebred and Tenmarq), quality had a tendency to be independent of protein content (the regression coefficients, though negative, were small), while in the other varieties quality tended to

decrease with an increase in protein content. The regression curves obtained by Sandstedt and Ofelt (1940) suggested a similar relationship; however, their data also indicated lower quality in samples having low protein content. The flours included in the present report were not of sufficiently low protein content to check this possibility.

Blending Values. It was suggested by Sandstedt and Ofelt (1940) that the baking results obtained by diluting with starch to a definite protein level might be an indication of the efficiency of the protein of the flours for blending purposes. On this basis, the proteins of Turkey, Nebred, and Tenmarq grown under this particular set of conditions were about equal in blending value and somewhat better than the other varieties. Differences in blending efficiency between varieties were greater in the higher protein ranges. If the loaf volumes obtained from these diluted flours are taken as an index of their blending efficiency, these results indicate that blending efficiency is not only a varietal characteristic but is to a large extent dependent on environment and in some varieties also on protein content. Chiefkan, as a variety, was low in volume; however, Chiefkan grown at A and at D, diluted to 10% protein, gave loaf volumes as large as, or larger than, Blackhull grown at the other localities and as large as Tenmarq and Nebred grown at E, C, F, and K.

Summary

Baking properties of hard winter wheats were found to be markedly affected by the environments in which the wheats were grown. All varieties from one locality showed high absorptions; from another locality, poor handling properties; from others, shorter or longer than normal mixing requirements; and from others, exceptional loaf volume potentialities. These variations from the expected behavior were largely independent of protein content.

Quality in some varieties seemed to be practically independent of protein content, but in other varieties quality decreased with increase in protein content.

Though the varieties tended to respond in a similar manner to environmental changes, the degree of response was determined by variety. Varieties with low loaf volume potentiality gave the least loaf volume response to changes in environment, while varieties with high loaf volume potentiality gave the greatest responses; similarly, varieties with the shortest mixing requirement gave the least response in mixing requirement to environmental change, while varieties with the longest mixing requirement gave the greatest response. Correspondingly, the differences in loaf volume between varieties were greatest in localities which produced the flours with the largest loaf

volume potentialities and the differences in mixing requirement between varieties were greatest in localities producing the longest mixing requirements.

Blending efficiency of the flour protein, while largely dependent on variety, may be materially affected by environment and in some varieties may decrease with increase in protein content.

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A MICROSCOPIC STUDY OF THE BEHAVIOR OF FATS IN CAKE BATTERS

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The basic structure of cake batters has long been a matter of speculation. The fat-air dispersion phenomenon, not to mention the fat-liquid emulsification state, has proved an intriguing subject to those interested in the fundamentals of cake baking. The common use of emulsifying agents in cake shortenings has increased the interest during the past few years. Because of the subject's complexity, much of the information published to date deals with theoretical considerations. Morris (1929) concluded that the air cells present in cake batter are surrounded by a film of sugar syrup and egg protein, or both, which in turn is emulsified in fat. Grewe (1937) reported that creamed mixtures of sugar, fat, and eggs represent a water-in-oil emulsion. On the bases of viscosity and electrical conductivity studies, Sunderlin and Collins (1940) concluded that thin batters are oil-in-water emulsions whereas thick batters are water-in-oil emulsions. Their data indicate that there is a gradual transition from one type of emulsion to the other. Morr (1939) made microscopical examinations of baked cakes and showed that the starch and fat of cakes are imbedded in or at the surface of the protein matrix. It was indicated that hydrogenated fats collect in clumps of fairly large size at the cake-air interface, that butter appears to be more finely divided than hydrogenated fats and appears to be distributed throughout the entire crumb, and that liquid fats seem to collect in pools at the cake-air interface with only a small portion distributed within the crumb. Lowe (1943) indicated that thin batters produced inferior cakes whereas viscous ones produced more desirable cakes. Lowe speculated, "If in the thin batters, the fat is largely dispersed as an oil-in-water emulsion and in the viscous ones as a water-in-oil emulsion, it is not impossible to have both types of emulsion in the intermediate batters. Not all of the fat may be emulsified." Lowe finally concluded that fats may possibly be dispersed in cake in several ways, such as an oil-in-water emulsion; a water-in-oil emulsion; films, pools, or lakes throughout the cake ingredients or adsorbed on the starch and protein of the crumb as monomolecular (or possibly multimolecular) films; at the cake-air interface; or by a combination of all or some of these.

Bailey and LeClerc (1935) supported the theory that a water-in-fat emulsion is formed during the creaming of cake batter.

Based on a microscopic examination of cake batter, Sunderlin and Collins (1940) concluded that gas bubbles were present in batter in grapelike clusters.

The work reported in this paper was undertaken to develop additional information regarding the basic structure of cake batters containing fats; to study the effect of emulsifying agents on cake batter dispersion; to determine the fate of suspended air cells during the baking process; to observe the structural changes taking place during baking; and to attempt to correlate known cake defects such as shrinkage and texture irregularities to some controllable factor or factors.

Materials and Methods

Cake types were both pound cake and white layer cake. In the case of pound cake, the percentage of fat was relatively high when compared with the white layer cake formula. The batter temperature was maintained at 75°F in all cases, unless otherwise stated.

After the batter was mixed, a minute portion was transferred to a glass slide and a cover glass was pressed down until the batter was extended sufficiently to become transparent to reflected light.

The fat was colored with fat soluble dyes, either yellow AB¹ or OB,² and consequently it became relatively easy to follow the behavior of the fat prior to and during baking. Observations were recorded by means of photomicrographs. Higher magnifications (up to 225 X) were of value in observing the behavior of single air spaces and single fat areas, while lower magnifications (25 X) were necessary for the study of several fat areas concurrently.

To permit a study of the changes which occur in the structure of the batters during baking, the microscope was specially equipped with a heating stage. The stage consisted of a resistance coil covered by a steel plate. The coil was operated from a 6-volt transformer and its temperature could be controlled, within limits, by means of a rheostat. Batters which were cooked or baked on slides by means of the heating stage were compared with similar batters taken from partially baked batter removed from cake layers during the regular process of baking.

The point at which the batters became "baked" was determined by means of polarized light. Upon the completion of the baking process, the characteristic birefringence of the starch granule disappeared. This loss of birefringence when a film of the batter was baked on the slide was confirmed with cakes baked in a normal manner.

¹ Benzeneazo naphthylamine—a certified dye used as a butter color.

² O-tolueneazo naphthylamine, also used as a butter color.

Results and Discussion

The Effect of Emulsifying Agents on Fat Dispersion. Both pound cake batters and white layer cake batters were made with hydrogenated shortening and with similar shortening containing varying percentages of mono- and di-glyceride emulsifying agent. Macroscopically the batters appeared "curdled" without the presence of emulsifying agent. Added quantities of emulsifying agent (up to 10% of the total shortening) overcame this curdled appearance and developed a thin but smooth batter. The viscosity of the batter decreased with each added quantity of emulsifying agent; the batter specific gravity increased, the cake volume increased, and the fat lake areas became more finely dispersed.

The findings of Grewe (1937) were verified. By means of electrical conductivity tests, it was proved that a water-in-fat emulsion existed during the creaming of fat, sugar, and eggs. It is difficult to attach much significance to the emulsion state at this particular stage of the cake making process. Just as soon as flour was added to this creamed mass the conductivity of the mixture increased almost to that of the emulsified aqueous medium. Apparently the major portion of the liquid (with its dissolved electrolytes) was released from its emulsified state by the addition of flour. It was evident that a continuous water-in-fat emulsion did not exist in the batters studied. Of course this does not eliminate the possibility of combination emulsions; *i.e.*, water-in-fat clumps distributed through an aqueous-flour medium.

Cake Batter at Varying Magnifications. The photomicrograph in the upper left corner of Figure 1 shows the structure of white layer cake batter as observed at 50 magnifications. The dark, irregular clumps or "lakes" are composed of fat, while the continuous field is made up of the aqueous phase with its dissolved sugar, salt, and baking powder, and its suspended flour and eggs. It will be noted that the air spaces are suspended only in the fat lakes.

The upper right photomicrograph is similar to that of the upper left corner, except that the magnification is greater. The air cells with their surrounding coating of fat are more clearly depicted. The flour particles suspended in the aqueous medium can be easily observed. No undissolved sugar and salt can be seen.

The lower left and right photomicrographs are also at 50 and 225 magnifications respectively. The flour particles have been stained by the addition of an iodine solution to more clearly depict their locations.

It is obvious from a study of the photomicrographs of Figure 1 that the fat is distributed through the batter in the form of small clumps or lakelike areas. The clumps of fat hold in suspension all of

the air which the batter contains. There is a complete absence of air spaces in the aqueous-flour areas. This is an important point and represents one about which there is considerable discord. It is also apparent that the fat does not represent the continuous phase of the emulsion.

Figure 2 shows a photomicrograph of white layer cake batter containing regular hydrogenated shortening without added emulsifying

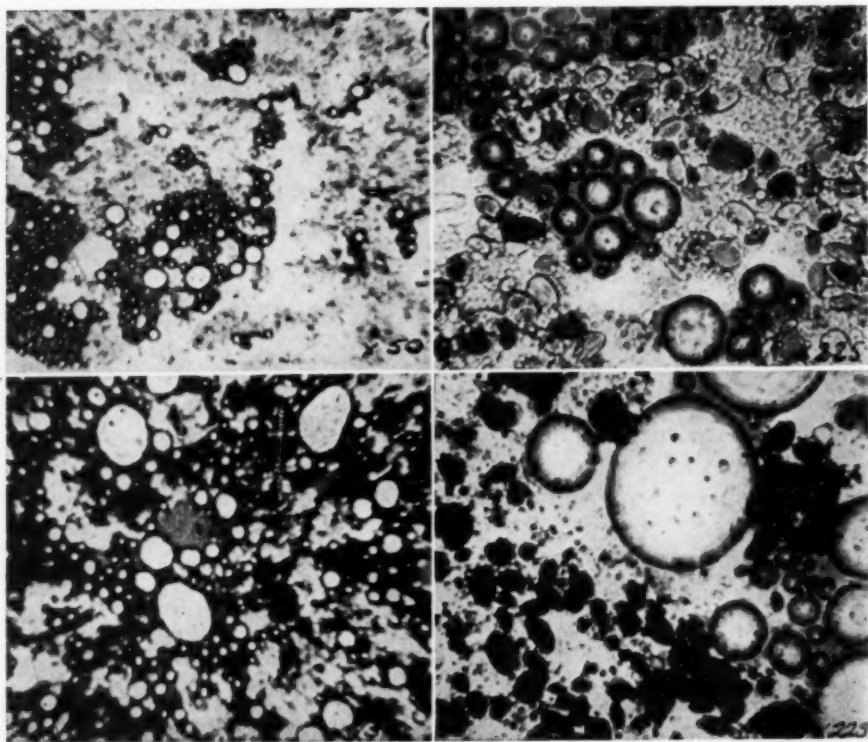


Fig. 1. Photomicrographs of white layer cake batter at varying magnifications. Upper left and right: 50 and 225 magnifications respectively. Lower left and right: 50 and 225 magnifications respectively (iodine added).

agent. The fat is distributed in the form of fat lakes or clumps and air spaces are present in each fat lake. Cakes baked from this batter shrank excessively upon baking, indicating a highly unstable cellular structure.

The shortening employed in preparing the batter for Figure 3 (upper) was the same as that for the batter of Figure 2, with the exception that 0.1% of a monoglyceride emulsifying agent was dissolved in the shortening. This resulted in the formation of more fat

lakes per unit area than were present in the batter which contained no added emulsifying agent. For the lower photomicrograph of Figure 3, the percentage of added emulsifying agent was increased to 0.25%. It can be readily seen that the additional emulsifying agent has resulted in a much larger number of fat lakes per unit area of batter.

The three photomicrographs at the left in Figure 4 indicate the progressive increase in the number of fat lakes per unit area with increases of emulsifying agent to 0.5%, 1%, and 3% respectively. Each increase in the quantity of emulsifying agent reduced cake shrinkage and resulted in cakes of generally superior quality.

The photomicrographs on the right in Figure 4 indicate the decreasing size of each fat clump with each increase in monoglyceride emulsifying agent. It is still evident that all air bubbles are sur-

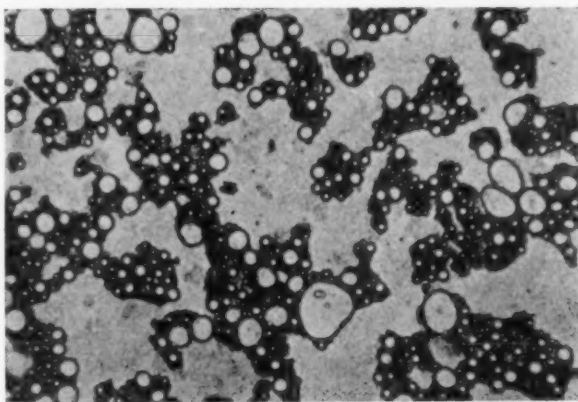


Fig. 2. Photomicrograph of white layer cake batter containing hydrogenated shortening (50 X).

rounded by fat and that few, if any, have entered the aqueous medium free from a protective fat coating.

With each addition of emulsifying agent, up to the level of 5 to 6%, there was a corresponding increase in cake volume. After the 5% level was reached, the cake volume approached a maximum, and at 8 and 9% the cake volume began to decline, indicating a detrimental effect when fat is dispersed in too fine a pattern.

The specific gravities of the batters became greater as the quantity of emulsifying agent was increased. There was a perceptible increase in specific gravity between 0 and 1%, with a gradual flattening out of the curve at the higher percentages. Batter viscosity was also checked and it was found that the batters containing emulsifying agent were less viscous than those made without.

The effect of increasing percentages of monoglycerides on the number of fat lakes per unit area and on batter viscosity is shown in Figure 5. The rate of change in viscosity is roughly inversely proportional to the rate of change in the number of fat lakes per unit area.

From this study sufficient data are not available to imply that there is a correlation between viscosity and the number of fat lakes

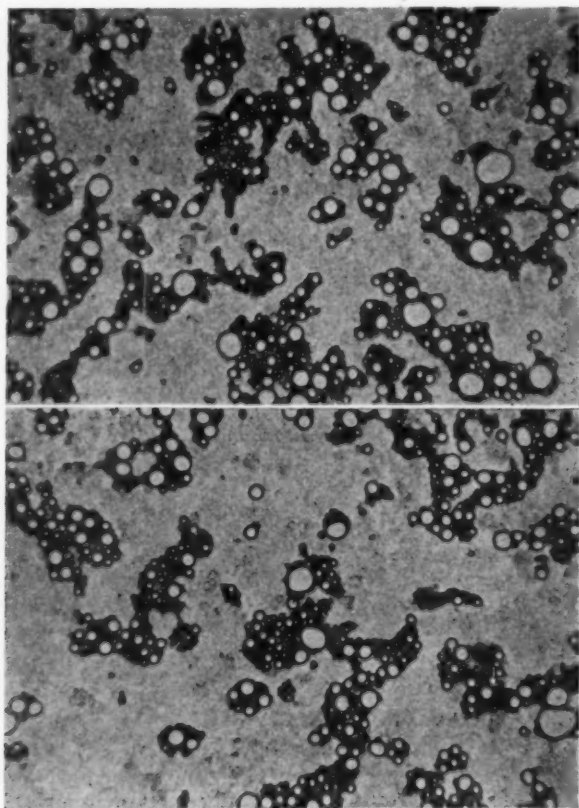


Fig. 3. Photomicrographs of white layer cake batter (50 X). Upper—0.1% monoglyceride emulsifying agent added. Lower—0.25% monoglyceride emulsifying agent added.

distributed through the batter; however, this appears to be an important factor. Since there is considerable discordant literature concerning the harmful effect of low viscosity batters on cake volume and other cake characteristics, it is only pertinent to point out that, within limits, the reverse of this contention is indicated, particularly if batters of lower viscosity are obtained by means of a finer fat dispersion.

Behavior of Lard in Cakes. Through photomicrographic studies it was indicated that lard disperses throughout cake batter in a much

finer pattern than that of hydrogenated vegetable shortenings. In fact, its dispersal pattern is somewhat similar to that obtained with hydrogenated fats containing emulsifying agents. There is one im-

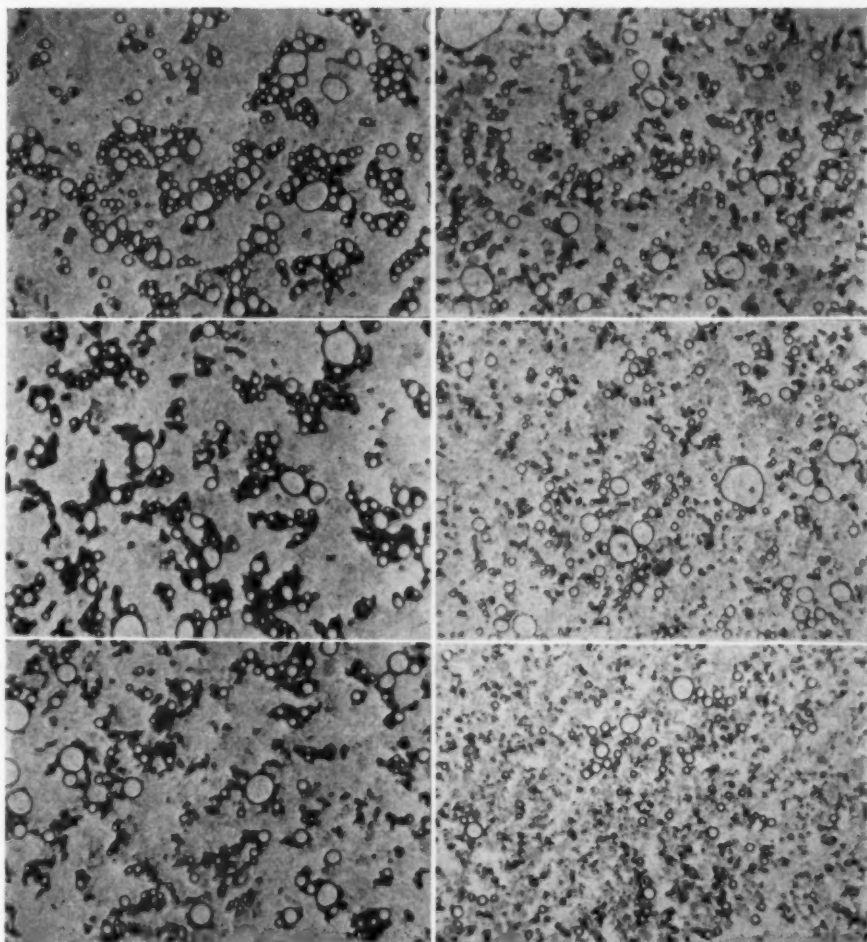


Fig. 4. Photomicrographs of white layer cake batter (50 X) showing the effect of increasing quantities of emulsifying agent in fat dispersion. Percentages given below refer to monoglyceride added.

Left upper..... 0.5%
Left middle..... 1.0%
Left lower..... 3.0%

Right upper..... 5.0%
Right middle..... 7.0%
Right lower..... 9.0%

portant difference: the quantity of air which is suspended in the fat lakes is reduced when lard is used as the shortening agent. This fine dispersion pattern explains why lard gives much better results in the richer type (140% sugar) layer cakes than does regular hydrogenated

shortening which contains no added emulsifying agent. Lard gives a fairly satisfactory performance in cakes of this type while hydrogenated shortenings without emulsifying agents produce failures. If the dispersal pattern normal to lard is changed through alteration of either its composition or texture to the point where the fat lakes in the batter become larger, the behavior of lard then approximates that of hydrogenated shortenings, and the ability to produce cakes of high sugar and moisture content without resorting to the use of emulsifying agents is thereby lessened. Conversely, the ability of lard to suspend air cells is increased when the fat dispersal pattern becomes coarse. Both air suspension and fat dispersal properties of lard can be regulated at any desired level.

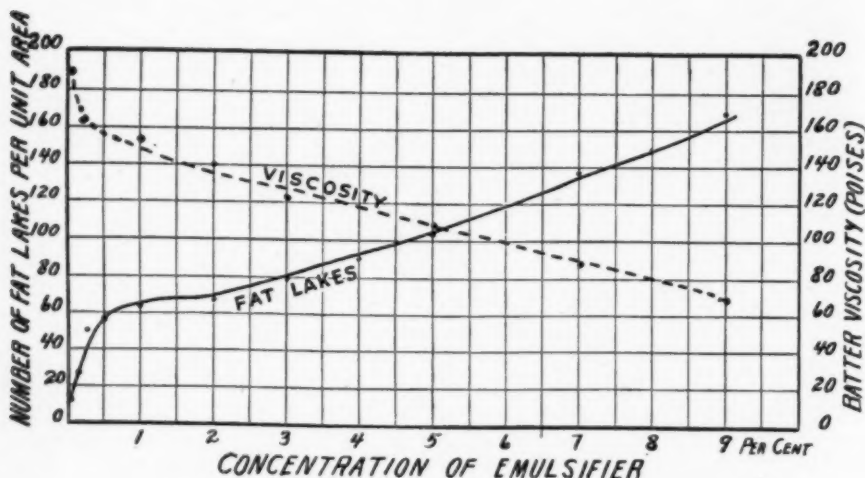


Fig. 5. Effect of concentration of monoglycerides present in shortening on the number of fat lakes and the viscosity of white layer cake batters.

The Behavior of Baking Powder in Cakes. A photomicrographic study of layer cake batters containing baking powder indicates that very few, if any, new gas cells are formed as a result of the baking powder reaction. The released carbon dioxide gas seems to collect at the air space interface, and each existing space seems to grow larger. It is significant that new air spaces are not formed. This observation further emphasizes the role played by creamed-in air cells in the expansion of cake batters during baking.

Observations of Batter Structure During Baking. Photomicrographs of batter made during the baking process are shown in Figure 6. After 5 min of baking had elapsed, it was found that the fat had melted and had released the air cells held in suspension. These cells were thereby

transferred from the fat phase to the aqueous medium. The fat, freed of its air bubble structure, collected in small lakes throughout the baking batter. Obviously these fat globules were smaller and in greater number when emulsifying agents were used. It is

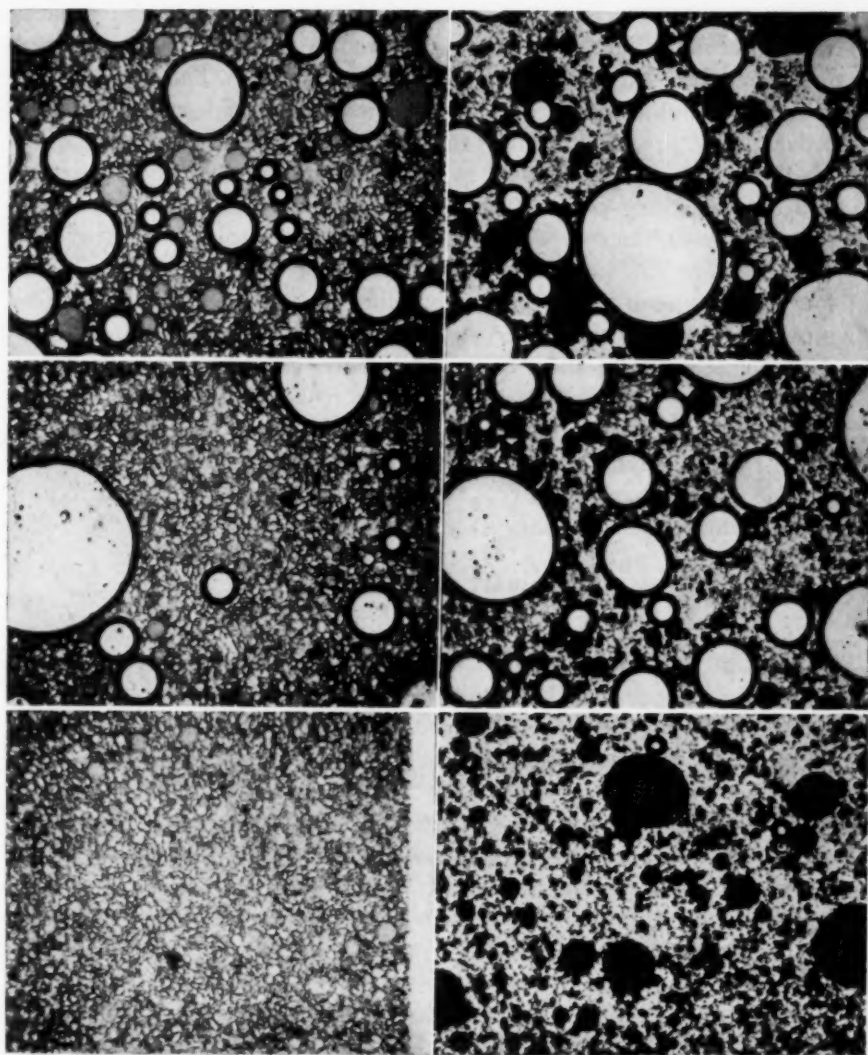


Fig. 6. Photomicrographs (100 X) of white layer cake batters made with and without emulsifying agent at various stages of the baking process. Upper, middle, and lower pairs represent 5-min, 10-min, and 15-min intervals respectively. In each pair, the picture at the left shows batter containing emulsifying agent; at right, without emulsifying agent. At the 5-min interval the batter was just beginning to expand; at the 10-min interval the batter had attained a high degree of expansion. After baking for 15 min it was impossible to transfer samples to the slide without loss of air; accordingly the photomicrographs for this time interval do not represent the actual batter conditions.

popularly believed that a greater distribution of fat, brought about by the use of emulsifying materials, will cause increased tenderness. Our work indicated that the reverse is true, because the cakes which contained no added emulsifying agents were far more tender than those containing the emulsifier.

As the batter continued baking, a movement of the air spaces was observed. The movement appeared to flow with the aqueous medium along the path of a convection current. The fat globules moved along the same path. There was little coalescence between separate fat areas. On the other hand, air spaces coalesced quite readily, as the larger cells often absorbed the smaller ones. As the end of the baking process was neared, a sudden increase in internal pressure was observed. This was reflected by rapid movement and distortions of the air spaces. Air spaces appeared to explode. The fat globules seemed to present points of extreme mobility. The movement during the period of high pressure was so vigorous that cake tunnels, holes, and other texture irregularities may be formed under certain conditions. It is even more significant that the phenomenon of shrinkage (which is partly overcome by the use of emulsifying agents) occurs at this stage of the baking process. The size of the fat globule possibly affects the stability of the cake structure and thereby influences cake shrinkage.

Summary and Conclusions

Layer and pound cake batters appear to be suspensions of air bubbles in fat distributed in a medium of flour and liquid. Little, if any, liquid appears to be emulsified in the fat. Soluble ingredients such as salt and sugar are dissolved by the water of the batter. The air spaces in layer and pound cake batters are invariably surrounded by fat.

During baking, the fat quickly melts and releases its suspended air to the flour-water medium. Gas produced by baking powder finds its way into the air spaces already existing within the batter. The completion of the baking process may be determined microscopically by means of polarized light. The cross pattern of wheat starch disappears at this stage.

There is a movement of air spaces at all times during the baking process. This movement appears to follow a definite convection pattern until the end of the baking process is neared. At this stage the movement becomes violent and without direction.

The use of monoglyceride type emulsifying agents produces a finer dispersion of fat throughout the cake batter.

Acknowledgment

The writer wishes to express appreciation to Mr. Harry Brody for his diligence in the preparation of numerous batches of cakes required by this study. In addition, acknowledgment is expressed to Drs. Lyle St. Amant and C. H. Koonz of the Histological Division for the vast amount of effort expended in the preparation of the photomicrographs. Without the cooperative effort of these individuals, this study would not have been possible.

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EFFECT OF SCAB ON THE QUALITY OF HARD RED SPRING WHEAT ¹

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Scab infections, caused chiefly by the fungus *Gibberella zeae* (Schw.) Petch (*G. saubinetii* Auct.), have resulted in substantial damage to hard red spring wheat in certain sections of Minnesota in recent years (Christensen and Rose, 1941). This damage has occurred chiefly in the south and west central sections of the state, but not to any appreciable extent in the northwestern part where a large portion of the spring wheat is grown. In 1941 and 1942 scab was a serious problem in the south central region, and in 1942 the infection spread to the west central part. In the latter year there was an average infection of 13% in the varieties of spring wheat grown in the one-fortieth acre plots at the Southeast Experiment Station at Waseca, and at the West Central Station at Morris. Four varieties of hard red spring wheat grown at the latter station for milling and baking tests for the Northwest Crop Improvement Association were so badly damaged with scab that they

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were considered to be unfit for comparative quality studies with varieties grown in other sections.

As a substantial quantity of scabby wheat is marketed, with little published information available regarding the effects of various proportions of scab-damaged wheat on physical and chemical characteristics or on milling and baking quality, a study was undertaken. The results are described in this paper.

Materials and Methods

Four hard red spring wheat varieties were included in this study: Thatcher (C.I. 10003),³ Regent (C.I. 12070), Ceres—Double Cross \times Ceres-Hope-Florence (Ns 2829,³ C.I. 12008), and Mercury² \times Comet-1018 (Ns 2822, C.I. 12071). The prevalence of scab was estimated in the standing grain before harvest and in the threshed grain. The former was derived by first estimating the average percentage of infected heads and multiplying this by the estimated percentage of infected florets in these infected heads. The infections for the four varieties so determined were 70, 60, 60, and 80%, respectively. Infection in the threshed grain was determined by examining each kernel in a sample of each variety, those being considered as scabby which had the characteristic grayish color. The percentages of infected kernels were found to be 16, 16, 12, and 20. These lower percentages are thought to be due to failure of many of the infected florets to produce grain and to removal of badly infected light-weight kernels by the fan and screen of the ordinary farm thresher.

The samples were divided into two lots designated as "sound" and "scabby." These were prepared by first blowing out as many of the light scabby kernels as possible with a blower, and then removing the remaining visibly damaged kernels by hand picking. The scabby wheat thus removed was composited to form the scabby sample. The sound samples contained no more than 1% of scabby grain and the scabby samples were at least 95% scabby.

The internal microflora of the sound and scabby samples was studied by means of a surface-sterilization technique followed by plating the wet kernels on sterile, acidified nutrient agar. Approximately 100 seeds were dipped in 70% ethanol, followed by immersion in mercuric chloride solution (1 : 1,000) for about 2 min. To minimize contamination with air-borne organisms, the kernels were then rinsed with dilute calcium hypochlorite solution and transferred to nutrient agar plates. Readings of the number and types of organisms arising from the seed were made after incubation for 7 days at room temperature. This technique is primarily designed for the determination of

³ "C.I." refers to accession number of the Division of Cereal Crops and Diseases, U.S.D.A.; "Ns" refers to North Dakota number.

fungi, but certain bacteria also grow under these conditions; bacteria and fungi other than *Gibberella*, *Helminthosporium* and *Alternaria* were not classified.

Determinations of test weight per bushel, moisture, protein, and ash were made on the wheats by the procedures given in Cereal Laboratory Methods (4th ed., 1941). Weight per 1,000 kernels was obtained. The apparent specific gravity of the wheats was determined by measuring, in a 25 ml burette, the volume of an ethanol-carbon tetrachloride mixture (1 + 1) displaced by a 5-g sample.

After scouring, the wheats were milled at a moisture content of 16.2% in a six-stand experimental mill. The weight of total flour recovered was employed in calculating flour yield; the patent flour (about 85% patent) was reserved for analytical and baking tests.

The patent flours were analyzed for moisture, protein, ash, total and reducing sugars, and carotinoid pigment content by the procedures given in Cereal Laboratory Methods (4th ed., 1941).

Experimental baking tests were made with the A.A.C.C. formula, using a National dough sheeter, low-form pans, and doughs scaled to 150 g. The flours milled from sound and scabby wheat for two of the varieties, Thatcher and Ns 2829, were baked in duplicate, employing combinations of the bromate levels (0, 1, and 3 mg potassium bromate per 100 g of flour), three mixing times (1, 2, and 4 min in a Hobart-Swanson mixer), and two fermentation periods (2 and 3 hr). Since in commercial practice scabby wheat would be milled in blends with sound grain, it seemed desirable to carry out baking tests on blends of the flours milled from sound and scabby wheat of the same variety. For each variety, blends containing 10 and 30% of flours milled from the scabby wheat were mixed with the corresponding flour from the sound wheat. These blends were baked in duplicate by the A.A.C.C. formula with the addition of 1 mg potassium bromate. The doughs were mixed for 2 min and fermented for two periods (2 and 3 hr).

As a measure of the effect of scab infection on the changes in dough consistency during fermentation, farinograph curves were made with doughs prepared according to the regular baking formula and containing 1 mg potassium bromate. These tests were confined to flours milled from the scabby and sound wheats obtained from Thatcher and Ns 2829. The doughs were mixed to minimum mobility, allowed to ferment in the farinograph for one hour, and then remixed for 10 min; after an additional hour of fermentation, the doughs were again remixed.

Results and Discussion

The internal microflora of the samples are given in Table I. Classification of the original samples into "sound" and "scabby" fractions

TABLE I
INTERNAL AEROBIC MICROFLORA OF SOUND AND SCABBY WHEATS

Variety and condition		Clean seed	Seeds infected with fungi and bacteria ¹				
			<i>Gibberella</i>	<i>Helminthosporium</i> sp.	<i>Alternaria</i> sp.	<i>Gibberella</i> , <i>Helminthosporium</i> and/or <i>Alternaria</i> ²	Miscellaneous fungi + bacteria
Regent	sound	% 2	% 1	% 45	% 54	% 9	% 7
	blighted	7	33	31	30	7	4
Thatcher	sound	30	0	19	46	1	5
	blighted	21	30	8	34	3	6
Ns 2822	sound	27	6	8	55	0	4
	blighted	18	38	4	37	2	3
Ns 2829	sound	40	3	10	43	0	4
	blighted	6	27	7	63	6	3
Mean	sound	25	2	20	50	2	5
	blighted	13	42	12	41	4	4

¹ The total percentages sometimes exceed 100% because some kernels yielded two kinds of fungi.

² The values in this column represent the percentages of seeds infected with two or more species.

on the basis of kernel color was quite satisfactory in separating the kernels which were diseased with *Gibberella*. Both classes of samples were, however, appreciably infected with other microorganisms, particularly *Helminthosporium* sp. and *Alternaria* sp. Since the extent of infection with microorganisms other than *Gibberella* is about the same in both series of samples, differences in chemical composition and in milling and baking properties may be attributed, in large part, to infection with *Gibberella*.

The test weight, weight per 1,000 kernels, flour yield, apparent specific gravity, and ratio of flour yield to apparent specific gravity are given in Table II.

TABLE II
PHYSICAL PROPERTIES AND FLOUR YIELD OF SOUND AND SCABBY WHEAT

Variety and condition		Test weight	Weight per 1,000 kernels	Flour yield	Specific gravity	Flour yield Specific gravity
		lb/bu	g	%		
Thatcher	sound	61	20.70	74.2	1.402	52.9
	scabby	56	16.30	73.1	1.378	53.0
Ns 2829	sound	62	36.24	76.2	1.381	55.2
	scabby	57	26.67	75.0	1.362	55.1
Regent	sound	61	31.48	78.1	1.389	56.2
	scabby	56	24.76	76.1	1.351	56.3
Ns 2822	sound	61	33.45	75.3	1.381	54.5
	scabby	52	22.59	73.5	1.346	54.6
Mean	sound	61.2	30.47	76.0	1.388	54.7
	scabby	55.2	22.58	74.4	1.359	54.8

The test weight of the scabby wheat averaged 6 lb less than that of the sound wheat. This difference would have been greater but for the fact that the scabby samples contained some kernels which were only slightly shrivelled but which were affected by scab, as shown by a gray color. Moreover, many of the very light, scabby kernels were lost during the threshing and cleaning operations; this would also occur in commercial practice. The variety Ns 2822 was most affected by scab and showed the greatest difference between the test weight of the sound and scabby wheat.

The weight per 1,000 kernels averaged 7.9 g lower for the scabby than for the sound wheat. Thatcher had the lowest and Ns 2829 the highest values; these varietal differences are a direct reflection of variations in the average size of the kernels.

The flour yield was slightly but consistently less for the scabby than for the sound wheat. It averaged 1.6% less. This is a smaller difference than would be expected from the lower test weight of the scabby wheat. It is, however, highly significant, statistically, as shown by a variance analysis of the data. The scabby wheat presented no particular difficulties in milling.

As expected, the specific gravity was lower for the scabby than for the sound grain. The ratios of flour yield to specific gravity were the same for sound and scabby wheat for any given variety but differed between varieties.

The chemical analyses of the wheats and patent flours are recorded in Table III. No consistent differences were found between the pro-

TABLE III
EFFECT OF SCAB INFECTION ON CHEMICAL COMPOSITION

Variety and condition		Wheat ¹		Patent flour ¹					
		Protein	Ash	Protein	Ash	Reducing sugar	Total sugar	Non-reducing sugar ²	Carotinoid pigment ³
		%	%	%	%	%	%	%	ppm
Thatcher	sound	13.0	1.52	12.3	0.44	0.17	1.05	0.88	2.64
	scabby	13.3	1.62	12.5	0.50	0.23	1.13	0.90	3.18
Ns 2829	sound	15.2	1.72	14.1	0.42	0.22	1.14	0.92	1.91
	scabby	14.8	1.82	13.6	0.53	0.34	1.15	0.81	2.51
Regent	sound	14.1	1.48	13.7	0.41	0.23	1.34	1.11	2.44
	scabby	14.3	1.54	13.8	0.50	0.32	1.32	1.00	2.88
Ns 2822	sound	14.9	1.42	14.4	0.40	0.20	1.03	0.84	2.52
	scabby	14.2	1.57	13.4	0.51	0.31	1.18	0.87	3.18
Mean	sound	14.3	1.54	13.6	0.42	0.20	1.14	0.94	2.38
	scabby	14.2	1.64	13.3	0.51	0.30	1.20	0.90	2.94

¹ Analyses are expressed on a 15% moisture basis.

² Difference between total and reducing sugars.

³ Expressed as carotene.

TABLE IV

MEAN LOAF VOLUMES OBTAINED IN DIFFERENTIAL BAKING TESTS WITH FLOURS
MILLED FROM SOUND AND SCAB INFECTED WHEAT

Mix- ing time	Potassium bromate	Variety					
		Thatcher		Ns 2829		Both varieties	
		Sound	Scabby	Sound	Scabby	Sound	Scabby
min	mg	cc	cc	cc	cc	cc	cc

FERMENTATION TIME—2 HR

1	0	673	693	755	682	714	688
	1	688	710	820	748	754	729
	3	675	745	943	793	809	769
	Mean	679	716	839	741	759	728
2	0	710	715	798	705	754	710
	1	780	810	915	808	848	809
	3	843	815	810	808	826	812
	Mean	778	780	841	774	810	777
4	0	795	798	788	695	792	746
	1	895	898	858	733	876	816
	3	808	830	790	735	799	782
	Mean	833	842	812	721	822	782
	Mean (2 hr)	763	779	831	745	797	762

FERMENTATION TIME—3 HR

1	0	658	675	765	730	712	702
	1	710	723	860	800	785	762
	3	653	658	825	743	739	700
	Mean	675	685	817	758	746	722
2	0	660	713	770	735	715	724
	1	783	760	883	813	833	786
	3	698	710	813	768	756	739
	Mean	714	728	822	772	768	750
4	0	720	710	765	670	742	690
	1	770	795	790	703	780	749
	3	603	625	660	660	632	642
	Mean	699	710	738	678	718	694
	Mean (3 hr)	695	708	792	736	744	722
	General mean	729	744	812	740	770	742

TABLE IV—(Continued)
ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	F
<i>Flours</i>	3	60,208	50.1†
Variety, V	1	74,029	61.6†
Scab, S	1	40,501	39.7†
V × S	1	66,093	55.0†
<i>Treatments</i>	17	23,098	19.2†
Fermentation period, F	1	79,101	65.9†
Mixing time, M	2	22,423	18.7†
Bromate level, B	2	58,349	48.6†
F × M	2	17,599	14.7†
F × B	2	32,192	26.8†
M × B	4	12,238	10.2†
F × M × B	4	872	—
<i>Flours × treatments</i>	51	3,517	2.9*
V × F	1	11,290	9.4†
V × M	2	49,918	41.6†
V × B	2	5,778	4.8*
S × F	1	5,078	4.2*
S × M	2	568	—
S × B	2	617	—
Remainder	41	1,201	—
Between duplicates	72	691	
Total	143		

* Exceeds 5% point by comparison with "remainder" mean square.

† Exceeds 1% point by comparison with "remainder" mean square.

tein content of the sound and scabby wheats, but the latter were slightly higher in ash content. The flours milled from the scabby samples were slightly but consistently higher in ash and reducing sugar content, and were considerably higher in carotinoid pigment content than the corresponding flours from the sound wheats.

The mean loaf volumes for the differential baking tests, conducted with flours milled from the sound and scabby wheats obtained from Thatcher and Ns 2829, are recorded in Table IV, together with a variance analysis of the data.

For both varieties combined, the mean loaf volume for all baking treatments of the flours milled from the scabby wheats was significantly lower by 28 cc than the corresponding mean for the sound wheat. However, the average loaf volume from the scabby wheat of Thatcher was 15 cc greater than from the sound wheat, while the average volume from the sound grain of Ns 2829 was 72 cc greater than from the scabby grain. The loaf volumes of the flours milled from the scabby and sound wheat were similarly influenced by variations in mixing time and bromate level and showed only slight differences in regard to the effect of varying fermentation times. These data indicate that while

scab infection may sometimes result in flours of lower strength, as measured by loaf volume, the mixing, fermentation, and oxidation requirements are not appreciably influenced.

Aside from loaf volume, it is of importance to consider the effect of scab infection on other baking characteristics. Flours from sound and scabby wheats required the same absorption to make a dough of the proper consistency at the time of mixing. Doughs made from flour of sound wheat had normal handling properties, whereas those of flours from scab infected grain had wet, sticky surfaces, collapsed readily on being removed from the fermentation bowls for punching, and were difficult to handle. The marked decrease in consistency of the doughs made with scab infected flour as fermentation progressed is illustrated by the farinograph curves shown in Figure 1. Loaves made from flours of scab infected grain of Thatcher proofed slightly higher and had very slightly less oven-spring than the loaves representing sound grain. Loaves representing scab infected grain of Ns 2829 proofed slightly less and had less oven-spring than loaves representing sound wheat of this variety. The crust color of loaves from scab infected flours was much darker than the crust of corresponding loaves from sound wheat flours. The crust was thicker, especially on the bottom, and tended to "cup" or pull away from the bottom of the pan. Loaves from Ns 2829 flour were slightly worse in this respect than loaves from Thatcher flour. The crumb color of the loaves from "scab infected" flour was dull and very yellow. It was improved by longer mixing, longer fermentation, or more bromate.

The mean loaf volumes obtained in the second series of baking tests, in which the flours from sound wheat were compared with blends containing 10 and 30% of flours of the same variety from scab-infected wheat, are recorded in Table V, together with an analysis of variance of the data. For all varieties and both fermentation periods combined, the mean loaf volumes for the flours containing 0, 10, and 30% flour from scabby wheat did not differ significantly. For the 2-hr fermentation period, the presence of flour from scabby wheat tended to increase loaf volume, whereas the reverse was the case when the doughs were fermented 3 hr.

The undesirable handling properties of the doughs made entirely of flour from scab infected wheat, and also the darker crust and inferior crumb color of the bread, were greatly minimized in the blends. Even when the blends contained 30% of flour from scabby wheat, the handling properties of the doughs were normal, and the color of the crust and crumb of the bread was influenced only slightly. The loaves of Ns 2829 were the only ones to show "cupping" on the bottom when "scabby" flour was included in the mixture.

From an agronomic standpoint wheat scab may cause serious economic loss to the grower. The small, heavily infected kernels would tend to be lost in commercial wheat cleaning operations and thereby

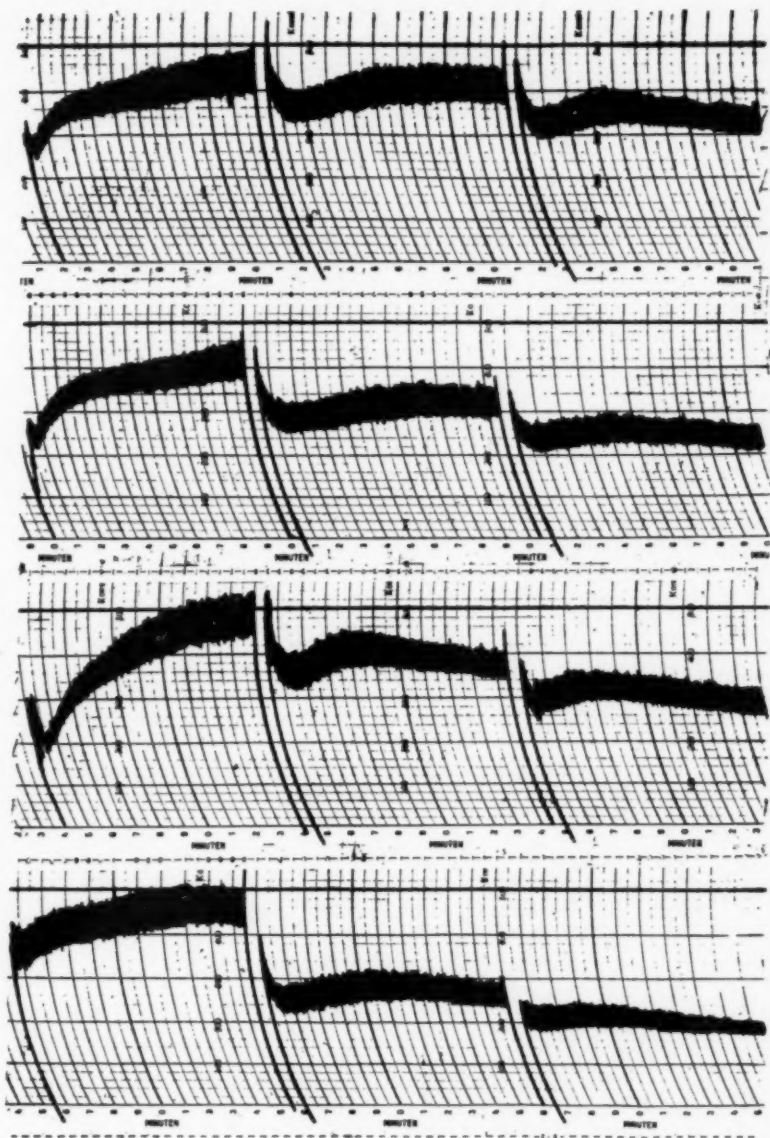


Fig. 1. Farinograms showing the influence of scab infection on the changes in dough mobility during fermentation. In each row, the first curve was obtained upon mixing the dough ingredients, and the second and third curves after one and two hr fermentation, respectively.

Top row—flour doughs representing sound Thatcher
 Second row—flour doughs representing scabby Thatcher
 Third row—flour doughs representing sound Ns 2829 #
 Bottom row—flour doughs representing scabby Ns 2829

TABLE V
MEAN LOAF VOLUMES FOR FLOUR BLENDS CONTAINING VARYING PROPORTIONS OF
FLOURS FROM SCABBY AND SOUND WHEAT OF THE SAME VARIETY

Flour from scabby wheat %	Loaf volume for variety ¹				All varieties
	Thatcher	Ns 2829	Regent	Ns 2822	
	cc	cc	cc	cc	cc
FERMENTATION PERIOD—2 HR					
0	743	813	705	743	751
10	738	870	743	803	789
30	763	830	763	893	812
All blends	748	838	737	813	784
FERMENTATION PERIOD—3 HR					
0	850	855	915	868	872
10	830	923	870	780	851
30	820	883	863	868	859
All blends	833	887	883	839	861
Mean	791	862	810	826	822

¹ A.A.C.C. formula with the addition of 1 mg potassium bromate per 100 g of flour.

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	F
Varieties, V	3	17,274	12.2†
Fermentation periods, F	1	56,033	39.6*
Blends, B	2	5,790	4.1
F × B	2	10,103	7.1*
V × F	3	10,875	7.7*
V × B	6	3,237	2.3
V × F × B (Error)	6	1,415	
Between duplicates	24	1,278	
Total	47	10,605	

* Exceeds 5% point.

† Exceeds 1% point.

reduce the yield of flour per bushel of wheat purchased to a much greater extent than is indicated in these studies; in addition, a somewhat shorter extraction would be necessary in order to produce flours of a given ash content than would be the case with sound wheat. A heavier bleaching treatment would doubtless also be required where scabby wheat is present in the mill mix. The strength of the flour from scabby wheat, as measured by loaf volume, and the oxidation, mixing, and fermentation requirements for optimum baking results

do not differ materially from sound wheat. Since the undesirable handling properties and the inferior crumb color of the bread are largely minimized when the flour from scabby grain is blended with that of normal wheat, scabby wheat does not present a serious problem from the baking standpoint.

Summary

The effect of scab, caused chiefly by the fungus *Gibberella zeae* (Schw.) Petch (*G. saubinetii* Auct.) on physical and chemical properties, milling, and baking value was studied with four hard red spring wheat varieties—Thatcher, Regent, Ns 2829, and Ns 2822, each of which was separated into sound (scab-free) and scab infected portions.

Scab infection markedly lowered test weight, weight per 1,000 kernels, and also decreased the apparent specific gravity of the kernels.

Flour yield was slightly but significantly decreased by scab infection. Ash and reducing sugar content of the flours milled from the scabby wheats were somewhat higher, and the carotinoid pigment content considerably higher, than that of the corresponding flours milled from sound wheat.

Differential baking tests with flours representing sound and scabby Thatcher and Ns 2829 showed that the absorption at mixing time, and the oxidation, mixing, and fermentation requirements of the flours from sound and scabby wheat were essentially similar. Doughs made with flours from the scab infected wheats had wet, sticky surfaces, collapsed readily, and markedly decreased in consistency as fermentation progressed. These doughs produced bread with a darker crust, and with a duller and more yellow crumb color than the corresponding doughs made with flours from sound wheat; also they exhibited a characteristic tendency to "cup" or pull away from the bottom of the baking pan.

These undesirable baking properties of flours from scabby wheat were greatly minimized when the flours were blended with those from sound wheat. The loaf volumes of blends containing up to 30% of flour from scabby wheat compared favorably with those for flours milled from sound wheat.

Acknowledgment

The authors are indebted to J. J. Christensen, Division of Plant Pathology, Minnesota Agricultural Experiment Station, for the determinations of the microfloral content given in Table I.

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THE EFFECT OF VARIATIONS IN CANADIAN SPRING WHEAT ON THE THIAMINE AND ASH OF LONG EXTRACTION FLOURS¹

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Several investigations of the effects of variety, soil, and climate upon the amount of thiamine in wheat have already been reported. It has been established that, in general, hard wheats are higher in thiamine than soft wheats (Downs and Cathcart, 1941; Sherwood *et al*, 1941). It has also been shown that environmental conditions have a very marked influence upon the thiamine content. Nordgren and Andrews (1941) found that the same variety grown in different locations exhibited great differences in thiamine. Recent studies of winter wheats by O'Donnell and Bayfield (1943) have confirmed the conclusions that thiamine content is influenced by variety, location, and climate. Seasonal variations were found to be considerable; the same varieties grown at the same stations were, on the average, 15% higher in thiamine in 1942 than in 1941. O'Donnell and Bayfield could find no significant correlation between thiamine and protein or between thiamine and wheat ash, but the authors believed that conditions which favored high protein and ash levels also tended to produce wheat high in thiamine.

Geddes and Levine (1942) showed that most of the thiamine of the wheat plant was present soon after blossoming and was thereafter translocated into the developing kernels. The mature kernels contained approximately 77% of all the thiamine in the plant.

Johannson and Rich (1942) conducted a survey of the 1940 Canadian spring wheat crop. Their results gave a mean value of 3.93 μg of thiamine per gram (1.78 mg/lb) with a standard deviation of 0.76 $\mu\text{g/g}$ and a range of 2.2 to 8.0 $\mu\text{g/g}$ (1.00 to 3.63 mg/lb). The means for the three Prairie Provinces showed no significant differences, but samples from Alberta were more variable. No correlation between thiamine and protein was found, though the fact that shrunken kernels were higher in thiamine than starchy kernels suggested that high protein crops would tend to be high in thiamine. They could not relate their thiamine results to soil types.

Studies of the 1939 and 1940 crops carried out by Whiteside and Jackson (1943) proved that the varieties of spring wheat grown in

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Western Canada were significantly different in thiamine content. A statistical analysis of their data also convinced these authors that "thiamine content was influenced by the same environmental factors that influenced protein, kernel weight, and bushel weight."

In Canada, where the sale of flour or bread enriched with synthetic vitamins is forbidden, the government has encouraged the consumption of a flour officially designated as "Vitamin B White Flour." At the end of August, 1943, the definition of this flour was amended to include a maximum dry-basis ash limit of 0.70%, as well as a minimum dry-basis thiamine limit of 400 I.U. per lb (2.64 μ g/g or 1.20 mg/lb). Though it is now known that variations in the thiamine content of wheats may not be reflected in the patent flours milled from those wheats, such variations do influence the thiamine of the long extraction flours (about 80%) advocated in official quarters in Canada.

Alcock (1943) has estimated that during the last nine months of 1942, Vitamin B White Flour constituted about 7% of the total volume of flour sold in Canada. Although the annual production of this flour is thus only about 700,000 bbl, its manufacture creates problems for the millers out of all proportion to the quantity involved, since it is made in small lots by mills scattered all over the country. Such operations are, in any case, notoriously difficult to control, and in this instance the difficulties are exaggerated by the opposing demands for low ash and high thiamine. The zone of safety between failure to meet the ash specification on the one hand, and failure to meet the thiamine requirement on the other, may be fairly wide in some mills and nonexistent in others. Differences in milling conditions and in mill equipment are factors, but one of the purposes of this paper is to show that the seasonal and local variations in the amounts of ash and thiamine in available wheat supplies exert an important influence on the problem of milling Vitamin B White Flour.

Aside from these very practical considerations, scientific interest in the natural variations in the thiamine content of wheat, their range, causes, and relationships to other characteristics of the grain, is far from exhausted. No thiamine data have yet been published for the Canadian spring wheat crop of 1941, and, accordingly, while our survey was limited in scope, a brief report of our results will precede the discussion of the problem of milling long extraction flours to ash and thiamine specifications.

Materials and Methods

Three hundred and eighty-three samples of spring wheat from points fairly well scattered over the three Prairie Provinces were analyzed for protein and thiamine. In addition, samples of Marquis and Thatcher

grown at five stations, and of Red Bobs grown at three of these stations, were supplied to us through the kindness of Dr. C. H. Goulden, Dominion Rust Research Laboratory, Winnipeg. These pure varieties and the products obtained from them by experimental milling were analyzed for thiamine and ash. All the samples were grown in 1941, a year which produced the highest protein crop on record.

Thiamine assays were made by the rapid method described by Hoffer, Alcock, and Geddes (1943), while the milling was carried out on a Buhler experimental mill.

Results and Discussion

Results of Thiamine Survey. Thiamine and protein results for the survey samples, grouped according to provinces, are summarized in Table I.

TABLE I
THIAMINE AND PROTEIN RESULTS BY PROVINCES FOR WHEAT SAMPLES
OF THE 1941 CROP

Province	No. of samples	Thiamine			Protein		
		Mean	Range	Std. dev.	Mean	Range	Std. dev.
		$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	%	%	%
Manitoba	93	4.61	3.0-5.5	0.50	14.59	12.4-17.6	1.03
Saskatchewan	182	4.60	2.9-6.3	0.57	15.57	12.2-17.6	0.92
Alberta	108	4.44	3.1-6.1	0.61	14.79	12.1-17.8	1.20
Total	383	4.56	2.9-6.3	0.57	15.11	12.1-17.8	1.13

Histograms showing the distribution of thiamine in all the samples, and for the samples segregated by provinces, are given in Figure 1.

The mean thiamine value for all samples was $4.56 \mu\text{g/g}$ (2.07 mg/lb), with a standard deviation of 0.57 and a range of 2.9 to $6.3 \mu\text{g/g}$ (1.32 to 2.86 mg/lb).

Samples from Manitoba and Saskatchewan had the same average thiamine value, though differing by 1% in protein content. Alberta samples were significantly lower in thiamine. Manitoba samples were the least variable, while samples from Alberta, as was found by Johansson and Rich (1942), displayed the greatest variability. Anderson and Eva (1943), on the basis of data covering a period of 12 years, report that wheat grown in Manitoba is the most uniform and Alberta wheat the most variable with respect to protein content. Such results are not unexpected, since in Alberta, wheat is grown over the widest range of latitude and elevation and on soils which show the widest variability, while in Manitoba the wheat-growing area is much smaller than in the other two provinces.

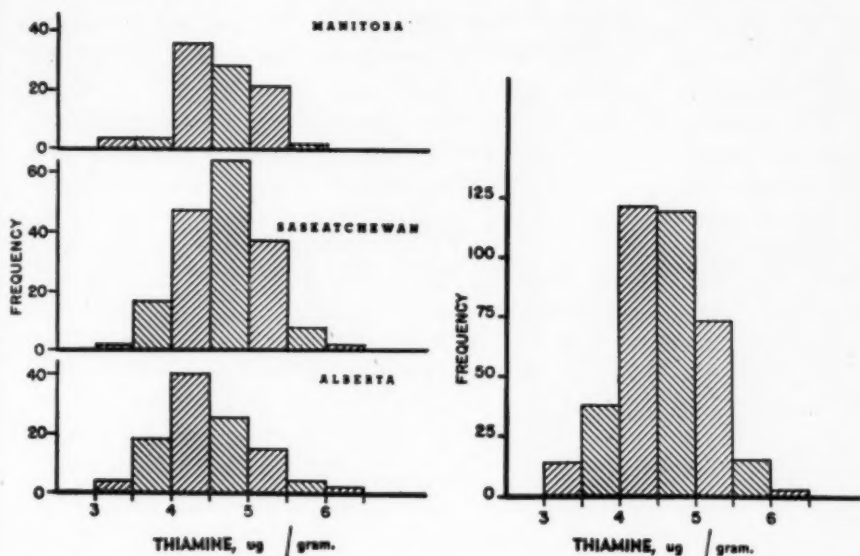


Fig. 1. Frequency distribution histograms for each province and for the three provinces combined, showing the number of samples falling within each $0.5 \mu\text{g/g}$ thiamine range.

A map showing the results of the thiamine survey is reproduced in Figure 2.

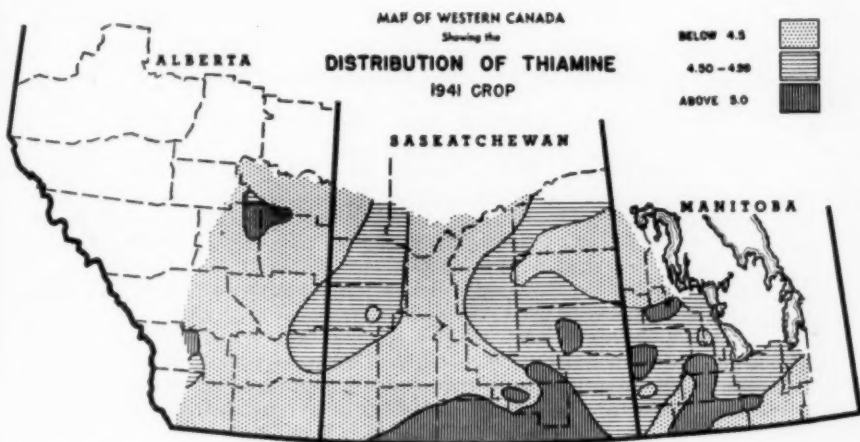


Fig. 2. Map of Western Canada zoned according to the thiamine values of survey samples, 1941 crop.

Because of the small number of samples in relation to the size of the wheat-growing area, the map is very considerably idealized, and it must be admitted that the boundaries of the thiamine zones were by no means as sharp as is indicated. The map should therefore be accepted as an illustration of the fact that samples of different thiamine

content were by no means randomly distributed but were roughly grouped into zones. The thiamine zones showed no relation to soil zones nor to the protein map for the season.

Thiamine and Soil Zones. The results for Saskatchewan and Alberta samples were classified according to the soil zones on which the wheats were grown, as shown in Table II.

TABLE II

THIAMINE AND PROTEIN RESULTS FOR SASKATCHEWAN AND ALBERTA SAMPLES OF THE 1941 CROP GROUPED ACCORDING TO SOIL ZONES¹

Soil zone	No. of samples	Thiamine			Protein		
		Mean	Range	Std. dev.	Mean	Range	Std. dev.
		µg/g	µg/g	µg/g	%	%	%
SASKATCHEWAN							
Black	56	4.50	3.2-6.0	0.51	15.65	12.2-17.6	0.89
Dark brown	59	4.74	3.3-6.3	0.66	15.85	14.2-17.0	0.72
Brown	59	4.59	2.9-5.8	0.56	15.25	13.2-17.4	1.01
ALBERTA							
Black	48	4.48	3.2-5.8	0.61	14.38	12.1-17.8	1.17
Dark brown	37	4.46	3.1-6.0	0.67	15.15	12.9-17.5	1.03
Brown	22	4.34	3.2-5.3	0.48	15.10	12.1-16.7	1.29
SASKATCHEWAN AND ALBERTA COMBINED							
Black	104	4.49	3.2-6.0	0.56	15.07	12.1-17.8	1.21
Dark brown	96	4.63	3.1-6.3	0.68	15.59	12.9-17.5	0.92
Brown	81	4.52	2.9-5.8	0.55	15.21	12.1-17.4	1.09

¹ Eight Saskatchewan and one Alberta sample came from the Gray Soil Zone and hence are not included in the above Table.

The following description of the three soil zones referred to in Table II is taken from a report by Joel *et al* (1936).

"In the southwestern section of the area (Saskatchewan) lies the Brown Soil Zone corresponding to the short-grass prairie region. In this zone the prevailing surface color of the soil is a light or drab brown. The relatively low moisture efficiency has allowed only a short thin cover of natural vegetation, with the result that the amount of organic matter is relatively low.

"The Dark Brown Soil Zone corresponds closely to the intermediate prairie region and the darker color of the surface soil reflects somewhat better moisture conditions and heavier vegetative cover of this region.

"The Black Soil Zone corresponds to the tall-grass prairie region and here the more humid climate and heavy grass growth give rise to dark-colored soils having the highest organic matter content to be found in the Province."

The Dark Brown Soil Zone of Saskatchewan produced wheat significantly higher in thiamine than the other soil zones. When, how-

ever, the results for the three soil zones in both provinces are studied, it becomes apparent that the data show no effect of soil zone on thiamine content. In spite of this, and of the fact that Johansson and Rich (1942) report a similar negative finding, it is probably too early to say that no relationship exists between thiamine and soil zone. A close concordance was found by Anderson and Eva (1943a) between the soil zone map of Western Canada and a protein zone map drawn on the basis of the results of surveys covering a period of 12 years. Nevertheless, in some of the individual years, the outlines of the soil zones could not be detected in the protein maps. More extensive thiamine surveys which reach into the Gray Soil Zone may still reveal a relationship between thiamine and soil zone. The small differences in the average protein levels of our samples from the three soil zones lead to the belief that our data do little to rule out the possibility of such a relationship. We suspect that it does exist, though of course the character of the soil itself may not be the determining factor but rather the continuing climatic differences of which the soil zones are, to a large extent, the consequence.

No explanation can be suggested for the relatively high standard deviation shown by the results of the thiamine determinations on samples from the Dark Brown Soil Zone, especially when it is considered in relation to the low standard deviation of the protein content in this zone.

Relation between Thiamine and Protein. The correlation coefficients between thiamine and protein for the three provinces and the three soil zones in Saskatchewan and Alberta are shown in Tables III and IV.

TABLE III

CORRELATION COEFFICIENTS BETWEEN THIAMINE AND PROTEIN BY PROVINCES FOR WHEAT SAMPLES OF THE 1941 CROP

Province	No. of samples	Correlation	1% Point
Manitoba	93	+0.306	0.266
Saskatchewan	182	+0.354	0.190
Alberta	108	+0.338	0.247
Total	383	+0.326	0.132

TABLE IV

CORRELATION COEFFICIENTS BETWEEN THIAMINE AND PROTEIN BY SOIL ZONES FOR SASKATCHEWAN AND ALBERTA WHEAT SAMPLES OF THE 1941 CROP

Soil zone	No. of samples	Correlation	1% Point
Black	104	+0.382	0.252
Dark brown	96	+0.350	0.262
Brown	81	+0.296	0.283

The correlation between thiamine and protein for each of the three provinces and for each of the three soil zones in Saskatchewan and Alberta was significant. The association between thiamine and protein is made still more evident by the arrangement of the data shown in Table V, where all the samples have been grouped according to protein content, and the calculated average thiamine content of each group is given.

TABLE V
THIAMINE VALUES FOR WHEAT SAMPLES OF THE 1941 CROP GROUPED
ACCORDING TO PROTEIN CONTENT

Protein	Thiamine values			
	Man.	Sask.	Alta.	Total
%	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Under 13.9	4.36	4.11	4.26	4.28
14 to 14.9	4.64	4.38	4.21	4.42
15 to 15.9	4.74	4.60	4.65	4.64
16 to 16.9	4.83	4.76	4.65	4.75
Over 17	—	4.98	5.20	5.05

A scatter diagram of thiamine on protein is shown in Figure 3.

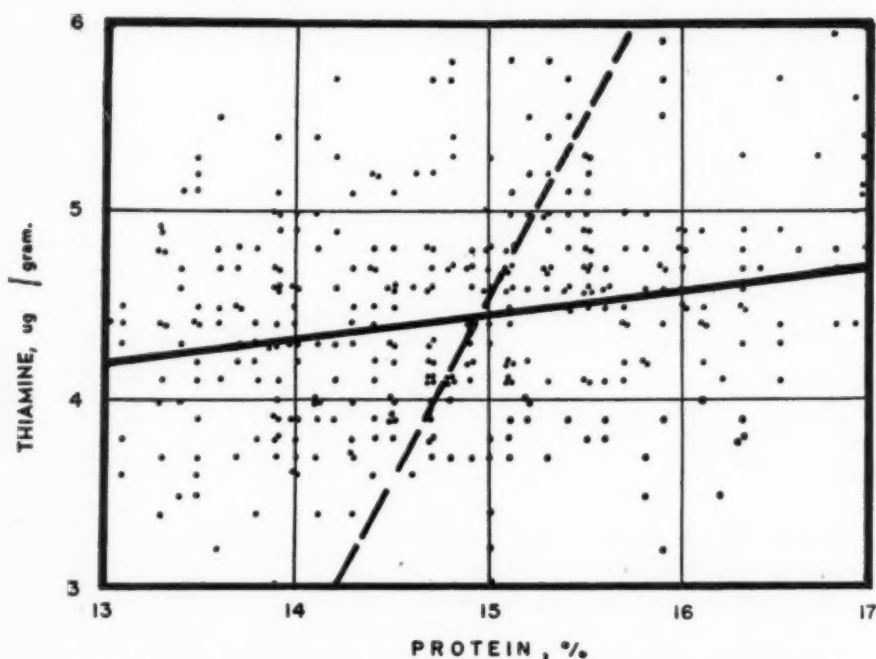


Fig. 3. Scatter diagram for thiamine and protein content in wheat samples, 1941 crop. The solid line is the regression line of thiamine on protein and the broken line the regression line of protein on thiamine. Correlation coefficient is $+0.326$.

Because the points are so widely scattered on the correlation surface, it is impossible to predict the thiamine content of a sample of country-run wheat from the amount of protein it contains. The protein in mill mixtures and shipments from terminal elevators, which consist of blends of wheats from many parts of Western Canada, should, however, afford a basis for the prediction of vitamin content.

Seasonal Variations in Wheat Thiamine. From the work of Geddes and Levine (1942) it is evident that after blossoming, there is a steady movement of thiamine from the stems, leaves, and glumes of the wheat plant to the growing kernels, with but little net gain in the amount of thiamine in the plant as a whole. In a year of low yield per acre, the plant thiamine is distributed over a smaller number of kernels, or the kernels are thin and shrunken because normal filling has not occurred. In either case, a relatively high thiamine content is to be expected in the seed. On the other hand, the work of O'Donnell and Bayfield (1943), to which reference has already been made, leads to the conclusion that the longer the filling and ripening period, the higher will be the test weight and the lower the thiamine content of the crop. A negative correlation is therefore to be expected between yield per acre and thiamine content. Up to the present, limited studies have been made of only three Western Canadian crops. As far as they go, these studies confirm the expected relationship, as is shown in Table VI.

TABLE VI
THIAMINE CONTENT AND YIELD OF THREE WESTERN CANADIAN WHEAT CROPS

Year	Mean yield	Thiamine
	<i>bu/acre</i>	$\mu\text{g/g}$
1940	18.5	3.93 ¹
1941	12.9	4.56
1942	28.6	3.45 ²

¹ Johansson and Rich (1942).

² Average of 236 samples tested in the laboratory of the Western Canada Flour Mills Co., Ltd.

Preliminary data for 1943, when the yield of wheat per acre was 16.7 bu, indicate a mean thiamine content between 3.9 and 4.6 $\mu\text{g/g}$ (1.77 and 2.09 mg/lb).

Relation between Thiamine and Ash. Under the latest regulations, the long extraction flour advocated by the Canadian government, called Vitamin B White Flour, must contain, on a moisture-free basis, not more than 0.70% ash and have a natural thiamine content of not less than 2.64 $\mu\text{g/g}$ (400 I.U. or 1.2 mg/lb). Previously, any product which met the legal definition for flour could be packed as Vitamin B White Flour, provided it carried the required amount of thiamine and this thiamine was derived from the wheat kernel. Since the ash

in flour is related to the ash content of the wheat milled (Sherwood and Bailey, 1928), it is evident that the ratio of thiamine to ash in available wheat supplies has become a matter of importance for Canadian millers with the adoption of an ash limit for Vitamin B White Flour.

The regulations referred to were only announced after the work described in this paper had been completed and no attention was given to the ash content of our survey samples. However, ash and thiamine determinations were made on a few samples of pure varieties grown at different stations in 1941, and since these results may now possess some current interest, they are recorded in Table VII.

TABLE VII
ASH AND THIAMINE VALUES AND RATIOS OF THIAMINE TO ASH FOR PURE WHEAT VARIETIES GROWN AT FIVE STATIONS

Station	Marquis			Thatcher			Red Bobs		
	Ash	Thiamine	T/A	Ash	Thiamine	T/A	Ash	Thiamine	T/A
	%	µg/g		%	µg/g		%	µg/g	
Brandon	1.69	4.46	2.64	1.66	4.33	2.61	—	—	—
Indian Head	1.75	4.17	2.38	1.66	4.46	2.69	—	—	—
Melfort	1.28	4.08	3.19	1.35	4.00	2.96	1.24	4.43	3.57
Edmonton	1.40	4.10	2.93	1.45	5.03	3.47	1.27	4.33	3.41
Lacombe	1.44	3.83	2.66	1.30	4.41	3.39	1.34	3.90	2.91

It is clear, even from our very limited data, that the ratio of thiamine to ash in wheat varies widely. A mill grinding such wheat as is represented by the samples from Lacombe, Edmonton, and Melfort might be expected to experience less difficulty in producing long extraction flours to meet thiamine and ash specifications than a mill using wheat represented by the Indian Head and Brandon samples. The Marquis and Thatcher samples for both groups were approximately the same in thiamine content, but in the first group, the ash content was definitely lower—1.37% as compared with 1.69%.

The results of experimental milling tests, using a Buhler mill, bore out these expectations. All the Marquis and Thatcher samples were milled, and ash and thiamine determinations were made on all the mill products, consisting of six flour streams, shorts, and bran from each milling. Regression curves for thiamine on ash, each based on the data for both samples from each station, are reproduced in Figure 4 (a). The fact that all the curves have approximately the same slope shows that the distribution of the total thiamine and ash throughout the kernels is very similar in all five pairs of samples. The low thiamine-ash ratio in the Indian Head and Brandon samples is not, therefore, counterbalanced by a more favorable distribution of thiamine in relation to ash in the kernels.

The problem of the miller is brought out, perhaps more clearly, by a somewhat different treatment of the data. From the ash and thiamine results on the mill products from the two groups of samples—Group (A), consisting of the six samples from Lacombe, Edmonton, and Melfort, and Group (M), the four samples from Indian Head and Brandon—the curves shown in Figure 4 (b) were calculated. In these curves the increase in the thiamine content is plotted against the increase in ash content which occurs as the extraction is lengthened. It is realized that if these two mixtures had been milled in a commercial mill instead of a Buhler, curves of different shape would probably have been obtained. Nevertheless, it is believed that the curves would

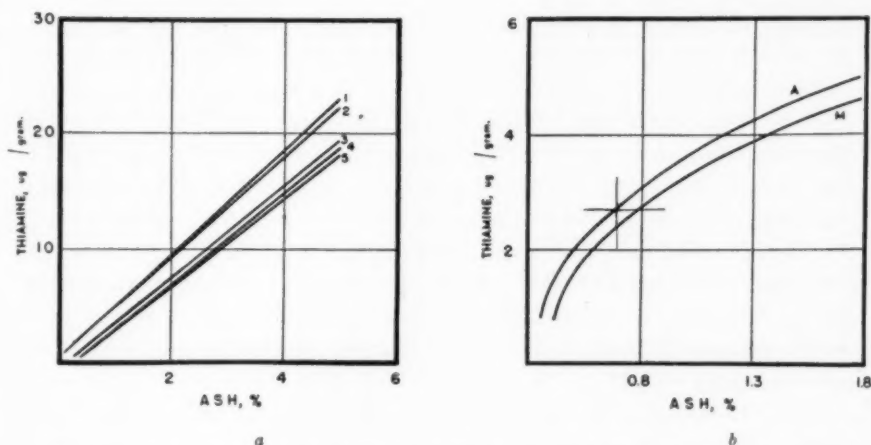


Fig. 4. (a) Curves showing the regression of thiamine on ash in Buhler mill streams, obtained by milling Thatcher and Marquis samples from the following five points: (1) Edmonton, (2) Melfort, (3) Lacombe, (4) Brandon, (5) Indian Head. (b) Curves showing the increase of thiamine with the increase in ash content as extraction is lengthened. The point for 0.70% ash and 2.64 μg of thiamine per gram is indicated. Curve (A) includes the samples from Lacombe, Edmonton, and Melfort. Curve (M) includes samples from Brandon and Indian Head.

still have been essentially parallel and that the height above the base line in the critical ash range, about 0.70% (moisture-free basis) as elsewhere, would have been significantly greater in one case than the other.

The territory in Western Canada devoted to wheat growing is large and some mills are obliged to draw their wheat supplies from limited areas. Our results indicate that in a mill grinding wheat having a low thiamine-ash ratio, whether that low ratio is due to low vitamin or to high ash, the production of long extraction flours defined with respect to both ash and thiamine may be a matter of great difficulty, or even an impossibility. Another mill, drawing its wheat from a different territory, may be able to meet the same specifications with ease. That

such differences do occur is already a matter of general commercial experience.

We, ourselves, have a detailed knowledge of only two mills drawing their wheat supplies from different areas. One of the mills is in Winnipeg and draws its supplies mainly from Saskatchewan and Manitoba. Over a period of several months this mill has produced Vitamin B White Flour containing, on a dry basis, 0.685% ash and 2.72 μg of thiamine per gram (1.23 mg/lb). When it is remembered that any decrease in ash requires a sacrifice in thiamine content, and any increase in thiamine can only be obtained by increasing the ash, it will be readily agreed by those who have had experience in mill control and are aware of the uncertainties of the thiamine determination, that these *mean* values indicate an inadequate margin of safety to take care of variations in operating results, sampling errors, and discrepancies in the results of different laboratories. It is scarcely necessary to say, therefore, that on some runs this mill produced flour which contained, at the same time, more than 0.70% ash and less than 2.64 μg of thiamine per gram (1.20 mg/lb). The other mill is located in Calgary and grinds mixtures made up entirely of Alberta wheat. During the same period, its Vitamin B White Flour averaged 0.675% ash and 2.88 μg of thiamine per gram (1.31 mg/lb). Here the margin of safety, though perhaps not adequate, is much wider than at Winnipeg, and according to the control laboratory's data, none of the Calgary flour failed to meet the specifications.

Five representative samples of the wheat mixtures from each mill were milled on the experimental mill. The shorts was divided into two portions by bolting on a 54GG sieve, so that nine samples were obtained from each milling. All 90 samples were analyzed for moisture, ash, and thiamine, and taking average results, the ash and thiamine values for products representing increasing percentages of the Winnipeg and Calgary wheats were calculated. The results are given in Table VIII.

These data support the view that the differences in the Vitamin B White Flour produced at the two mills were due to differences in the wheat. Calgary wheat was higher in thiamine and somewhat lower in ash and, when experimentally milled under the same conditions, yielded, at every level of extraction, products possessing a higher thiamine-ash ratio than Winnipeg wheat. Wheat mixtures showing much wider differences in thiamine-ash ratio could undoubtedly be found in other mills in Western Canada, and it would thus seem that any legal maximum ash limit, for flours milled to meet a minimum natural thiamine requirement, must either be so low as to work a

TABLE VIII

THIAMINE¹ AND ASH¹ RESULTS FOR BUHLER EXPERIMENTAL MILL PRODUCTS FROM COMMERCIAL WHEAT MIXTURES²

Winnipeg wheat mixtures				Calgary wheat mixtures			
Extraction	Ash	Thiamine	T/A	Extraction	Ash	Thiamine	T/A
%	%	µg/g		%	%	µg/g	
43	0.51	0.77	1.51	40	0.50	0.79	1.58
59	0.53	1.04	1.96	56	0.51	1.05	2.06
70	0.58	1.57	2.71	69	0.55	1.60	2.91
73	0.60	1.61	2.68	71	0.55	1.64	2.98
78	0.71	2.46	3.46	77	0.66	2.56	3.88
83	0.94	3.10	3.30	82	0.90	3.40	3.78
100	1.82	4.45	2.45	100	1.77	4.84	2.73

¹ Ash and thiamine are on dry matter basis.² Short extraction flours from the Buhler mill are relatively high in ash and thiamine.

hardship on some mills, or so high as to provide no control over the color and other grade characteristics of the flour.

Summary

Thiamine values for 383 samples of hard red spring wheat grown in Western Canada in 1941 ranged from 2.9 to 6.3 µg/g (1.32 to 2.86 mg/lb) with a mean of 4.56 µg/g (2.07 mg/lb).

Alberta samples were significantly lower and more variable in thiamine content than Saskatchewan or Manitoba samples.

A significant positive correlation between protein and thiamine was found.

A negative correlation between mean yield per acre and average thiamine content of Western Canadian wheat crops was indicated by a study of the available data for three seasons.

The relationship of thiamine content to ash content varies widely in wheats grown in different localities. These variations are reflected in the thiamine-ash ratios of long extraction flours, with the result that some commercial mills have considerably more difficulty than others in producing such flours within specified ash and thiamine limits.

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THE EFFECT OF SOME WETTING AND REDUCING AGENTS ON THE MIXING TIME AND ON THE QUALITY OF BREAD¹

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It is generally accepted by bakers that good quality bread will result only when the mixing time employed closely approaches the optimum. The optimum mixing time of a particular flour may be too long or too short to fit the shop schedule. Also, a flour with short mixing requirements may be too sensitive to overmixing and a flour with long mixing time calls for a large expenditure of energy. It would therefore be desirable if the optimum for the flour could be changed to meet shop conditions. This investigation was made to study some of the effects of changing the optimum mixing time, by use of various agents, on the quality of bread from flours differing in their normal mixing requirements.

¹ Contribution No. 105, Department of Milling Industry.

Review of Related Investigations

Swanson and Andrews (1942) showed that surface active agents, notably Aerosol OT (sodium dioctylsulfosuccinate), increased the mixing time. They suggested that these agents caused a certain type of protein denaturation which allowed greater inter- or intramolecular penetration of water. Such modification of the gluten proteins should influence the baking characteristics. They also demonstrated (1943) that surface active agents influence principally the proteins. These authors (1944) further presented some baking data which indicated that the use of Aerosol OT and cysteine (cysteine monohydrochloride) to change the mixing requirement did not injure the loaf volume or texture of bread.

The reduction of the time required to mix a dough to minimum mobility by compounds containing free -SH groups has been demonstrated by several workers. Jørgensen (1936) presented farinograms and baking data showing that 0.1% glutathione greatly affected the physical properties of dough and also had a harmful action on bread quality. Yeast water had similar effects. Potassium bromate tended to reduce the harmful effects. Jørgensen (1936) and Balls and Hale (1936) stated that glutathione activated latent proteolytic enzymes. Later, Balls and Hale (1936a) suggested that glutathione did not activate the enzymes but rather activated the gluten proteins, thus making the proteins more sensitive to enzymatic attack.

Sullivan, Near, and Foley (1936) found that fresh wheat germ and also its water-soluble fraction reduced the time required to mix a dough to minimum mobility but injured the baking quality. Later, Sullivan, Howe, and Schmalz (1936) isolated glutathione from the germ extract and found that this reduced the mixing time and quality of the baked loaf in a similar manner to the glutathione prepared from yeast. Potassium bromate tended to reduce the harmful effects of glutathione.

Freilich and Frey (1939) showed that cysteine and glutathione produced very harmful effects in bread and also that these could be partly overcome by mixing in oxygen. Ziegler (1940, 1940a) demonstrated that KBrO_3 oxidized the GSH (glutathione) form to the GSSG form and that this oxidation proceeded slowly in a fermenting dough.

Swanson (1940) demonstrated by mixograms (Swanson and Johnson, 1943) that cysteine decreased the mixing time and increased the rate of breakdown. Swanson and Andrews (1944) showed that cysteine and other reducing agents changed the mixogram patterns and that this was proportional to the molecular concentration of these agents. The similarities of mixogram patterns obtained with cysteine and other agents containing -SH groups suggest that these groups are

the causative components. Sodium chloride tended to minimize the effects of cysteine.

Ofelt and Larmour (1940) have also shown that cysteine reduced the mixing time and that the effect was greater with increasing amounts of cysteine. Cysteine did not cause any significant change in loaf volume, but an improvement in the texture was noted. They expressed the view that the action of cysteine was a colloidal effect on the gluten rather than an activation of enzymes.

General Plan of Investigation

Materials. Tenmarq with a normal long mixing requirement and Chiefkan with a shorter mixing requirement were the varieties chosen for these studies. Both flours had 12.6% protein and the flour ash was 0.43% for Tenmarq and 0.45% for Chiefkan. Both flours were composited from several smaller samples of experimentally milled flour. Data given later show that the mixing time of the Tenmarq flour was about normal for that variety, while for Chiefkan flour it was about 1 minute longer than usual for that variety.

Methods. Two baking formulas were employed: namely, the malt-phosphate-bromate (MPB) formula of Aitken and Geddes (1934) and a rich formula which included 6% dry milk solids (Ofelt and Larmour, 1940). The Swanson-Working type mixer used had a speed of 98 rpm. The baking absorption for each flour was 64%. A standard fermentation of 3 hours and a 55-minute proof was used with both formulas. The loaves were baked at 425°F for 25 minutes. Loaf volumes were measured immediately upon removal from oven. Crumb color and texture scores were made the next day after baking. All loaf volumes are the averages of duplicate bakes.

The variables were: (1) substances which influence the mixing time; (2) their amounts; (3) the duration of mixing; and (4) varying amounts of potassium bromate. Two surface active agents were employed, Aerosol OT and sodium lauryl sulfate. Three reducing agents were employed: cysteine; boiled yeast extract, prepared according to the method of Jørgensen (1936); and H₂S-saturated water, prepared by bubbling hydrogen sulfide through water. All solutions were made on the day of baking and added to the other baking ingredients in the mixing bowl.

Experimental Results

Results from MPB Formula. The effects of wetting and reducing agents on baking results obtained with the malt-phosphate-bromate formula and optimum mixing time are presented in Table I.

TABLE I

EFFECTS OF WETTING AND REDUCING AGENTS ON BAKING RESULTS, EMPLOYING THE MPB FORMULA AND OPTIMUM MIXING TIME

Treatment	Conc.	Mixing time	Loaf volume	Grain texture	Crumb color	Dough characteristics at pan stage
		min	cc	%	%	

TENMARQ						
Check	%	4.0	885	75-o	85cy	Bucky
Aerosol OT	0.06	4.5	845	73-o	85cy	Bucky
	0.12	5.8	820	70-o	85cy	Bucky
	0.24	8.0	750	65-o	85cy	Very bucky
Sodium lauryl sulfate	0.04	4.0	838	78-o	85cy	Bucky
	0.08	4.5	755	70-o	83cy	Soft
	0.16	4.5	625	60-o	80cy	Soft
Cysteine	0.005	2.0	950	83-o	85cy	Very good
	0.010	1.5	950	83-o	85cy	Very good
	0.020	1.2	900	85-o	85cy	Very good
Boiled yeast extract	ml					
	3	2.7	953	80-o	83cy	Very good
	6	2.0	920	75-o	80cy	Very good
	12	1.8	873	65-o	78cy	Soft
H ₂ S-saturated water	3	3.5	890	80-o	80cy	Soft and elastic
	6	3.0	885	82-o	80cy	Soft and elastic
	12	3.0	893	82-o	80cy	Soft and elastic

CHIEFKAN						
Check	%	2.3	800	75-o	80cy	Puttylike
Aerosol OT	0.06	3.0	793	77-o	80cy	Puttylike
	0.12	4.0	805	77-o	80cy	Puttylike
	0.24	5.5	780	75-o	82cy	Puttylike
Sodium lauryl sulfate	0.04	2.3	670	70-c	80cy	Puttylike
	0.08	2.3	715	65-c	80cy	Soft
	0.16	2.2	635	60-c	80cy	Soft and sticky
Cysteine	0.005	2.0	815	77-o	82cy	Soft
	0.010	1.6	810	77-o	82cy	Soft
	0.020	1.5	795	75-o	82cy	Soft
Boiled yeast extract	ml					
	3	2.0	780	77-o	80cy	Soft
	6	1.7	750	72-o	80cy	Soft and sticky
	12	1.5	700	60-o	80cy	Very soft
H ₂ S-saturated water	3	2.0	775	78-o	80cy	Soft
	6	1.8	795	77-o	80cy	Soft
	12	1.6	770	77-o	80cy	Soft

The mixing times used were those needed to produce doughs of optimum consistency. The addition of increments of Aerosol OT gradually lengthened the mixing time to double that of the check. Sodium lauryl sulfate had only a small effect on the mixing time. Increments of cysteine made the mixing times of Tenmarq and Chiefkan almost the same. Thus the reduction for Tenmarq, for which the mixing time is normally long, was relatively much greater than for Chiefkan, for which the mixing time is normally short. The effects of boiled yeast extract and H_2S -saturated water were similar to cysteine.

The effects on baking results from the wetting and reducing agents were variable. Sodium lauryl sulfate was unsatisfactory for both flours. This agent had an unfavorable effect on the physical properties of the dough. Increasing the amounts of Aerosol OT produced baking results which were progressively poorer than the checks for Tenmarq and about equal to the checks for Chiefkan. Cysteine produced marked improvement in loaf volumes, textures, and dough-handling properties of Tenmarq, but for Chiefkan, cysteine had less effect. Boiled yeast extract in the smaller amounts produced results similar to cysteine for Tenmarq. For Chiefkan, increasing amounts of this extract were distinctly detrimental. The H_2S -saturated water produced no marked effect with either flour.

The data indicate that when an agent such as Aerosol OT increased the mixing time for Tenmarq, the results were unsatisfactory, and when the agent such as cysteine decreased the mixing time, the effects were beneficial. Chiefkan was less responsive to these treatments. The treatment of Tenmarq with Aerosol OT, which caused an increase in mixing time, produced pronounced buckiness of the dough similar to that obtained by an excess of certain oxidizing agents.

Results from Rich Formula. Various percentages of Aerosol OT and of cysteine together with 2, 3, or 4 mg of potassium bromate were used with the rich formula. Both optimum and constant mixing times were employed. The results from Tenmarq are presented in Table II, and those from Chiefkan in Table III. The figures in Table II show the following: (1) the effect of potassium bromate was minor or obscured by the other substances; (2) Aerosol OT with optimum mixing time produced bread with larger loaf volumes and with equally good textures as compared to the checks; (3) with constant mixing time, Aerosol OT had no apparent effect on the quality of the bread; (4) cysteine with optimum mixing time produced baking results somewhat better than the checks; (5) with constant mixing time, the results with cysteine were increasingly poorer than the checks. The doughs were soft and sticky with very poor handling properties, indicating serious over-mixing.

TABLE II

EFFECTS OF OPTIMUM AND CONSTANT MIXING TIME WITH VARIOUS CONCENTRATIONS OF AEROSOL OT, CYSTEINE, AND POTASSIUM BROMATE ON BAKING RESULTS FOR TENMARQ FLOUR

Treatment	Conc.	KBrO ₃	Mixing time	Loaf volume	Texture grain	Crumb color	Row in Fig. I
	%	mg	min	cc	%	%	
OPTIMUM MIXING TIME							
Check		2	4.0	883	88-o	85cy	1
		3	4.0	878	88-o	85cy	1
		4	4.0	815	80-o	85cy	1
Aerosol OT	0.06	2	5.0	938	85-o	86cy	1
	0.06	3	5.0	978	85-o	86cy	1
	0.06	4	5.0	925	82-o	86cy	1
	0.12	2	6.0	973	90-c	88cy	1
	0.12	3	6.0	925	90-c	88cy	1
	0.12	4	6.0	925	85-o	88cy	1
	0.24	2	8.0	885	86-o	85cy	1
	0.24	3	8.0	918	86-o	90cy	1
	0.24	4	8.0	930	86-o	88cy	1
CONSTANT MIXING TIME							
Aerosol OT	0.06	2	4.0	865	87-o	85cy	2
	0.06	3	4.0	893	87-o	85cy	2
	0.06	4	4.0	900	87-o	85cy	2
	0.12	2	4.0	890	85-c	84cy	2
	0.12	3	4.0	890	85-c	84cy	2
	0.12	4	4.0	870	87-c	84cy	2
	0.24	2	4.0	825	83-o	80cy	2
	0.24	3	4.0	873	83-o	80cy	2
	0.24	4	4.0	850	83-o	80cy	2
OPTIMUM MIXING TIME							
Cysteine	0.005	2	3.0	940	85-c	80cy	3
	0.005	3	3.0	873	85-c	80cy	3
	0.005	4	3.0	900	82-c	80cy	3
	0.010	2	2.0	905	85-c	80cy	3
	0.010	3	2.0	905	83-c	80cy	3
	0.010	4	2.0	918	80-c	80cy	3
	0.020	2	1.5	853	83-o	80cy	3
	0.020	3	1.5	868	82-o	80cy	3
	0.020	4	1.5	863	80-o	80cy	3
CONSTANT MIXING TIME							
Cysteine	0.005	2	4.0	868	81-o	80cy	4
	0.005	3	4.0	878	81-o	80cy	4
	0.005	4	4.0	870	80-o	80cy	4
	0.010	2	4.0	865	81-o	80cy	4
	0.010	3	4.0	860	82-o	80cy	4
	0.010	4	4.0	873	80-o	80cy	4
	0.020	2	4.0	798	81-o	80cy	4
	0.020	3	4.0	830	82-c	80cy	4
	0.020	4	4.0	815	80-c	80cy	4

TABLE III
EFFECTS OF OPTIMUM AND CONSTANT MIXING TIME WITH VARIOUS CONCENTRATIONS
OF AEROSOL OT, CYSTEINE, AND POTASSIUM BROMATE ON BAKING RESULTS
FOR CHIEFKAN FLOUR

Treatment	Conc.	KBrO ₃	Mixing time	Loaf volume	Texture grain	Crumb color	Row in Fig. 2
	%	mg	min	cc	%	%	
OPTIMUM MIXING TIME							
Check		2	3.0	783	80-o	85cy	1
		3	3.0	780	80-o	85cy	1
		4	3.0	795	87-c	88cy	1
Aerosol OT	0.06	2	3.5	793	82-o	85cy	1
	0.06	3	3.5	785	83-o	85cy	1
	0.06	4	3.5	835	88-c	88cy	1
	0.12	2	4.0	823	80-o	83cy	1
	0.12	3	4.0	808	86-c	85cy	1
	0.12	4	4.0	818	89-c	88cy	1
	0.24	2	5.0	808	85-o	85cy	1
	0.24	3	5.0	870	87-c	85cy	1
	0.24	4	5.0	803	87-c	85cy	1
CONSTANT MIXING TIME							
Aerosol OT	0.06	2	3.0	778	85-c	85cy	2
	0.06	3	3.0	803	85-c	85cy	2
	0.06	4	3.0	805	85-c	85cy	2
	0.12	2	3.0	800	85-c	83cy	2
	0.12	3	3.0	820	85-c	83cy	2
	0.12	4	3.0	805	80-o	82cy	2
	0.24	2	3.0	795	85-c	80cy	2
	0.24	3	3.0	828	82-o	80cy	2
	0.24	4	3.0	850	80-o	80cy	2
OPTIMUM MIXING TIME							
Cysteine	0.005	2	2.0	748	82-o	83cy	3
	0.005	3	2.0	800	85-c	83cy	3
	0.005	4	2.0	793	83-c	82cy	3
	0.010	2	1.5	775	80-o	82cy	3
	0.010	3	1.5	775	83-o	82cy	3
	0.010	4	1.5	750	80-o	82cy	3
	0.020	2	1.3	695	75-o	78cy	3
	0.020	3	1.3	720	75-o	78cy	3
	0.020	4	1.3	675	75-o	78cy	3
CONSTANT MIXING TIME							
Cysteine	0.005	2	3.0	710	80-o	82cy	4
	0.005	3	3.0	765	80-o	82cy	4
	0.005	4	3.0	765	80-o	82cy	4
	0.010	2	3.0	695	80-o	80cy	4
	0.010	3	3.0	700	80-o	80cy	4
	0.010	4	3.0	715	80-o	80cy	4
	0.020	2	3.0	625	70-o	75cy	4
	0.020	3	3.0	640	70-o	75cy	4
	0.020	4	3.0	660	70-o	75cy	4

It appeared that the longer mixing time resulting from Aerosol OT, even in the presence of the larger amounts of potassium bromate, caused no deleterious oxidation of the dough. This contrasts with the results obtained with the MPB formula and presented in Table I. It is probable that the presence of milk in the rich formula acted as a buffer in minimizing the effects of oxidation consequent upon long mixing. The buffering action of milk was shown by Ofelt and Larmour (1940). When cysteine was present, the 4-minute constant mixing time was too long and produced deleterious effects.

The data for Chiefkan in Table III show the following: (1) the loaf volumes and textures are on a lower level than those presented in Table II, because of the poorer quality of Chiefkan as compared with Tenmarq; (2) no consistent improvement was noted with the use of potassium bromate; (3) Aerosol OT with both optimum and constant mixing time produced better baking results than the checks; (4) the cysteine treatment produced poorer results than the Aerosol OT treatment; (5) with the optimum mixing time, the results were distinctly better than for the constant time.

The handling properties of dough are difficult to describe adequately because they have no accepted standards of measurement. It was noted that when the mixing time of Tenmarq was reduced with cysteine, the dough-handling properties at the pan stage remained very good. When the time had been increased with Aerosol OT, the doughs were bucky. This was more noticeable with the MPB formula which had no milk. The addition of Aerosol OT did not improve the handling properties of the dough, whereas cysteine made the dough more soft and sticky than the checks.

The photographs of the loaves from Tenmarq corresponding to the data in Table II are presented in Figure 1 and similarly the loaves from Chiefkan (Table III) are shown in Figure 2. The four groups of loaves in each figure are arranged in the same order as the four groupings in the tables. The horizontal placings of the loaves in the figures from left to right are in the same order as the vertical arrangements of the data in the tables. The general groupings of the loaves are further elucidated by the legends for each of the figures. Thus by counting the loaves from left to right in any row and the figures in the tables in any group vertically, comparison can be made between the appearance of any loaf and the baking data.

The appearances of the bread obtained from optimum and constant mixing times, as influenced by the addition of Aerosol OT or cysteine, do not differ markedly from the two sets of three check loaves to the left in each figure. The more accurately measured effects of the treatments are indicated by the data given in the tables.

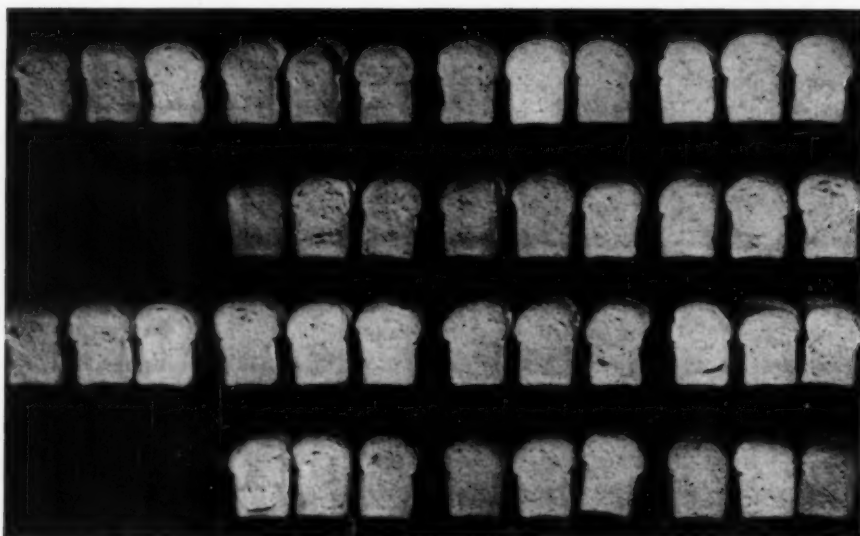


Fig. 1. Effects of optimum and constant mixing time with various concentrations of Aerosol OT, cysteine, and potassium bromate on baking results for Tenmarq flour.

- Row 1. Aerosol OT—Optimum mixing time.
- Row 2. Aerosol OT—Constant mixing time.
- Row 3. Cysteine—Optimum mixing time.
- Row 4. Cysteine—Constant mixing time.

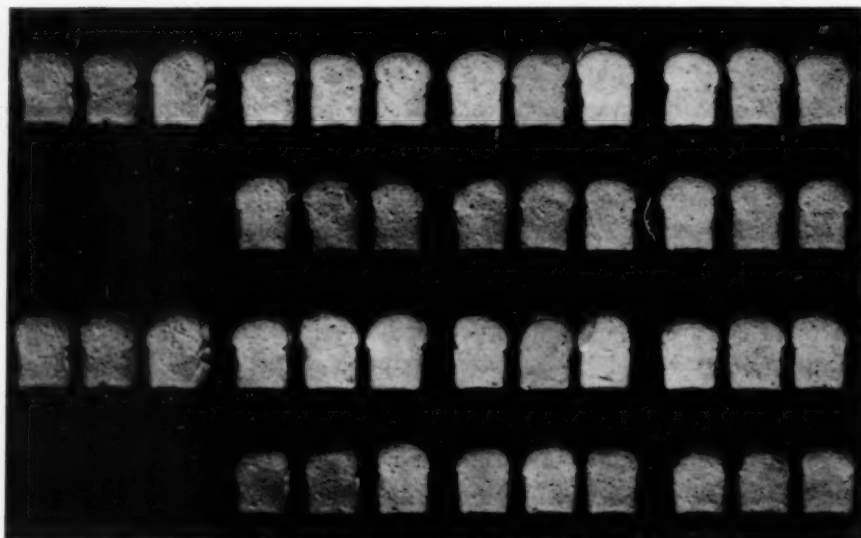


Fig. 2. Effects of optimum and constant mixing time with various concentrations of Aerosol OT, cysteine, and potassium bromate on baking results for Chiefkan flour.

- Row 1. Aerosol OT—Optimum mixing time.
- Row 2. Aerosol OT—Constant mixing time.
- Row 3. Cysteine—Optimum mixing time.
- Row 4. Cysteine—Constant mixing time.

Discussion and Summary

This baking study was made to test the effect on baking of changing the optimum mixing time of two wheat varieties, Tenmarq and Chiefkan, by using surface active and reducing agents. The surface active agents employed were Aerosol OT and sodium lauryl sulfate. The reducing agents employed were cysteine, yeast water, and H_2S -saturated water. Two formulas were employed, the MPB and a rich formula which included 6% dry milk solids. With the rich formula, the treatments were limited to Aerosol OT and cysteine with varying amounts of potassium bromate.

The results obtained with the MPB formula were different from those obtained with the rich formula, showing that the constituents in the formulas had an influence on the action of the agents used. Sodium lauryl sulfate showed less desirable effects than Aerosol OT. With the MPB formula, Aerosol OT made the dough of Tenmarq bucky, whereas Chiefkan became puttylike. Cysteine with the MPB formula and with a greatly reduced mixing time produced doughs with very good handling properties and improved bread for Tenmarq. With Chiefkan, cysteine made the doughs soft and produced no improvement in the bread.

The effects of boiled yeast extract and H_2S -saturated water were similar to those from cysteine, indicating that the effective component which shortens the mixing time is the -SH group. Potassium bromate, used only with the rich formula, had small or inconsequential effects. Increasing amounts of potassium bromate did not correct the harmful effects of the larger amounts of cysteine on Chiefkan.

The amounts used of these various agents were critical. When beneficial results were obtained with small amounts, larger quantities often produced harmful effects. Superior results were obtained by using the mixing time which coincided with the point of minimum mobility as affected by the various agents added. The results for this optimum mixing time were consistently better than for the constant mixing time as established by the checks.

Tenmarq was, on the whole, more favorably responsive to these treatments than Chiefkan. No treatments made the characteristics of Chiefkan similar to those of an untreated Tenmarq. Making the mixing time of Tenmarq shorter was beneficial, while shorter mixing time for Chiefkan had deleterious effects. Increasing the mixing time of Chiefkan produced a slight improvement in the bread.

These studies indicate the possibilities of using a surface active agent to increase the mixing time and still obtain good bread. This is conditioned on the use of a suitable formula with proper amounts of

the agent and optimum mixing time. The possibilities are also indicated of using a reducing agent to shorten the mixing time of a flour which normally has a long mixing requirement and in this manner obtained bread of good quality. This is also conditioned by the use of a suitable formula, proper amounts of the agent, and optimum mixing time.

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EFFECT OF VARIETY AND ENVIRONMENT ON SOME QUALITIES OF MALTED WHEAT FLOUR¹

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Investigations of malted wheat have recently been reported by Geddes, Hildebrand, and Anderson (1941) and by Kneen and Sandstedt (1942). The latter paper contains extensive references to earlier work, and a further review of the literature seems unnecessary. It is now generally agreed that the utility of malt supplements depends on their alpha-amylase activity and that this varies widely in barley, wheat, and rye malts. No study has yet been made of the difference in the alpha-amylase or other enzymatic activities of malts made from different varieties of hard red spring wheat or from the same variety grown under different conditions; and this matter appeared to merit investigation.

Materials and Methods

Materials. The materials consisted of samples of 23 varieties of hard red spring wheat grown in 1941 under strictly comparable conditions at 16 stations in Western Canada. The varieties are listed in Table I. They represent several named varieties and new crosses which were being studied by Canadian plant breeders and cereal chemists in that year. The stations, also listed in Table I, are fairly widely distributed throughout Western Canada, with five in Manitoba, six in Saskatchewan, and five in Alberta.

From these individual samples two sets of composite samples were prepared, namely, (1) a set of 23 composites, one for each variety, made up by combining equal quantities of wheat from each station, and (2) a set of 16 composites, one for each station, made up by combining equal quantities of each variety.

The varietal composites showed some variation in both bushel weight and protein content. Mean values and ranges were: bushel weight, 64.7 lb, and 63.5 to 66.0 lb; protein content, 15.6%, and 14.9 to 16.2%. The ranges for station composites were 62.5 to 66.5 for bushel weight, and 12.3 to 17.4% for protein content. However, when the lowest value (Fallis, 12.3%) is disregarded, the station range for protein content becomes 14.4 to 17.4%. The high protein values were caused by the dry growing season in 1941. It is unfortunate

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TABLE I

INTERVARIETAL AND INTERSTATION RELATIONS BETWEEN ALPHA-DEXTRINOGENIC ACTIVITY OF AND GAS STIMULATION BY MALT FLOURS

Variety	Gas stimulation	α -dextrinogenic activity	Station	Gas stimulation	α -dextrinogenic activity
	ml	units		ml	units
1. Garnet	163	83	1. Swift Current, Sask.	160	55
2. Red Bobs	161	61	2. Edmonton, Alta.	158	57
3. H44×Reward	160	54	3. Gilbert Plains, Man.	158	53
4. R.L. 625×Mercury, I	158	56	4. Fallis, Alta.	158	51
5. Pilot	153	54	5. Broadacres, Sask.	156	44
6. Marquis×(H44×Marquis), II	152	48	6. Saskatoon, Sask.	155	52
7. Thatcher×Regent	150	49	7. Morden, Man.	154	52
8. Merit 3	150	48	8. Melfort, Sask.	154	51
9. H44×Thatcher, I	150	44	9. Scott, Sask.	154	50
10. Marquis×(H44×Marquis), III	149	45	10. Lacombe, Alta.	154	48
11. Regent Selection I	148	45	11. Winnipeg, Man.	154	48
12. Regent Selection II	148	43	12. Brandon, Man.	151	47
13. Thatcher	146	51	13. Indian Head, Sask.	150	40
14. Red Thatcher	146	36	14. Beaverlodge, Alta.	149	43
15. C.A.N. 1926	145	35	15. Lethbridge, Alta.	148	37
16. H44×Thatcher, II	144	40	16. Portage la Prairie, Man.	140	41
17. Regent×Canus, I	144	37			
18. Renown	142	49			
19. Apex	139	46			
20. Marquis×(H44×Marquis), I	138	46			
21. Marquis	136	41			
22. Regent×Canus, II	134	32			
23. Regent	130	44			

that the materials were not more representative of the average quality of wheat grown in Western Canada.

Wheat Flour. The wheats were milled to a yield of 70% in an Allis-Chalmers mill. The flours had an average protein content of 14.9%, and an average ash content of 0.45%.

Malting. Duplicate 350-g samples of each composite were malted as follows: steep to 44% at 50°F in equipment described by Anderson and Meredith (1940); germinate and grow for five days at 54°F in equipment not yet described but similar to that of Anderson (1937); and kiln for 24 hours with continuous rise in temperature from 85 to 120°F from 0–6 hours, and a uniform temperature of 120°F from 6–24 hours, in kiln described by Anderson and Rowland (1937). The samples were malted in four successive series, namely, first replicates of variety composites, first replicates of station composites, second replicates of variety composites, and second replicates of station composites; and the order of the samples was randomized within each series.

Malt Flour. The malts were milled to a yield of 75% in an Allis-Chalmers mill. The malt flours had an average protein content of 15.2% and an average ash content of 0.76%.

Analytical Methods. Protein, ash, gas production (volumetric method; 25 g flour, 6-hour fermentation), Lintner value (diastatic activity), and alpha-dextrinogenic (alpha-amylase) activity, were determined by the standard procedures given in "Cereal Laboratory Methods." Saccharogenic activity was determined by the method of Kneen, Beckord, and Sandstedt (1941). Total Lintner value and total saccharogenic activity were determined by making the original extracts of the flour or malt flour with 1% papain solution as outlined by Sallans and Anderson (1938). The total gas production of the wheat flour was determined by the volumetric method with the addition of papain equivalent to 0.5% of the weight of the flour. The gas stimulation by the malted wheat flour is reported as the increase in the gas production (volumetric method) of a standard base flour which results from the addition of 0.2% of the malt flour. Protein, ash, and gas production are reported on a 13.5% moisture basis, and all other values are given on a dry basis.

Results and Discussion

The alpha-dextrinogenic activity of the malt flour and its ability to stimulate gas production in a standard dough were studied with both the varietal and station composites. For these properties the intervarietal and the interstation relations can be examined. Data on the remaining properties relate to varietal composites, and illustrate only the intervarietal relations.

Alpha-dextrinogenic Activity and Gas Stimulation. The data on these two properties of the malt flour for both varieties and stations are given in Table I. The varieties and stations are listed in decreasing order with respect to gas stimulation.

Under the conditions used in the investigation, 0.2% of malt flour was added to a standard base flour having a gas production of 252 ml in 6 hr. The best variety, Garnet, increased the gas production by 163 ml to 415 ml; the poorest variety, Regent, increased the gas production by 130 ml to 382 ml. It is thus apparent that the range for varieties is comparatively small.

With respect to the station data, the range for gas stimulation is still lower. It is 20 ml over all stations, and if the lowest value is neglected it amounts to only 12 ml. While it is probable that the station composites do not represent as wide a range of environmental conditions as might occur in other seasons, the data certainly suggest that the effect of environment on the gas stimulation by malt flours

is not large. In this connection it may be noted that the Fallis sample, which had the lowest protein content, 12.3%, has essentially the same gas-stimulating activity as the Saskatoon sample, which had the highest protein content, 16.8%. These two samples must certainly have been grown under widely different conditions.

The data for alpha-dextrinogenic activity show greater differences between varieties. Garnet again stands first with an activity of 83 units, and this is more than twice the value for the poorest variety, No. 22, which has an activity of only 32 units. Among the stations the maximum spread is considerably smaller; Edmonton with 57 units, gave the highest value, and Lethbridge with 37 units, gave the lowest.

A relation between gas stimulation and alpha-dextrinogenic activity for both the varietal and station composites is clearly indicated in Table I. The correlation coefficients are 0.71 for varieties and 0.79 for stations, and both are well above the 1% level of significance. Kneen and Sandstedt (1942)—and several earlier workers—have already established the existence of this relation; but it has not previously been shown that it occurs both within and between varieties.

Both our correlation coefficients are appreciably lower than that (0.94) reported by Kneen and Sandstedt. Although theoretical explanations of the difference can be offered, the available data are hardly adequate to support an argument. It should be noted, however, that a correlation of 0.94 suggests that increased gassing power is almost wholly dependent on alpha-amylase activity, and that other factors are of little concern; and this led Kneen and Sandstedt to suggest that determinations of added gassing power and alpha-amylase "appear to be equally reliable for evaluating malts." The data given in the present paper do not support this hypothesis. However, we do not wish to be thought too dogmatic in our interpretation of the available correlation coefficients; it is clear that there is a fundamental relation between alpha-amylase activity and gas stimulation, but it is perhaps a moot question as to how close the relation actually is.

Investigation of causal relations by the correlation method is fraught with many difficulties. An association between two properties may exist in a series of samples produced by progressively changing the processing methods, merely because the change affects both properties in a uniform manner; such an association does not prove a causal relation. An association between two properties may be demonstrated with a set of samples of different varieties grown in the same environment, but may not occur in samples of one variety grown in different environments, or *vice versa*. Under these conditions, with pairs of properties such as gas stimulation and alpha-amylase, failure to obtain a correlation with both varietal and station (environmental)

composites leads to the conclusion that no close causal relation exists. However, when a correlation is found with all three sets of samples—samples produced by different methods, different varieties grown in the same environments, and one variety grown in different environments—there is every reason to believe that a fundamental causal relation exists. It is hazardous to base conclusions on the study of only one kind of samples, or on the study of a miscellaneous collection of samples of unrecorded history.

Effect of Papain Extraction. It is well known that the free saccharogenic activity of unmalted barley is low by comparison with the total saccharogenic activity determined by means of a papain extract, and that the total activity is correlated with the saccharogenic activity of the malted barley. These relations were investigated for wheat flour and the corresponding malted wheat flour of different varieties.

In the wheat flour, the free saccharogenic activity was found to be about 10% of the total saccharogenic activity. The mean values over all varieties were 3.1 and 32.6 units. The free activity is of little interest and it does not seem necessary to record the individual values for each variety. Data for total saccharogenic activity are given in Table II, and are discussed in the following section.

TABLE II
VARIETAL MEANS FOR SACCHAROGENIC ACTIVITIES AND GAS PRODUCTION

Variety No.	Malt flour			Wheat flour		
	Sacch. activity	Beta sacch. act.	Alpha sacch. act.	Total sacch. act. (Papain)	Total Lintner value (Papain)	Gas production
	units	units	units	units	° Lintner	ml
1	33.2	29.1	4.1	38.0	292	292
13	29.9	27.4	2.5	41.6	328	237
7	28.2	25.7	2.5	42.1	325	238
18	28.1	25.7	2.4	40.6	316	258
2	27.7	24.7	3.0	33.5	264	301
14	24.6	22.9	1.7	37.6	296	217
19	23.9	21.6	2.3	38.9	302	273
9	23.3	21.1	2.2	35.2	266	240
21	22.4	20.4	2.0	36.8	289	248
3	22.1	19.5	2.6	32.6	256	268
12	21.4	19.2	2.2	29.3	220	265
11	21.1	18.9	2.2	30.3	278	260
16	20.8	18.8	2.0	30.6	229	275
23	19.9	17.7	2.2	30.5	224	265
5	18.4	15.7	2.7	30.0	225	239
10	17.5	15.3	2.2	27.0	204	232
6	17.3	14.9	2.4	26.8	200	238
4	16.8	14.0	2.8	28.5	214	272
17	16.7	14.9	1.8	29.0	214	254
20	15.6	13.3	2.3	26.9	200	228
22	15.5	13.9	1.6	28.7	216	252
8	15.2	12.8	2.4	27.4	206	269
15	13.8	12.1	1.7	27.1	206	242

The gas production and total gas production (papain) of the wheat flour were also examined. The mean values over all varieties were 261 and 272 ml. Thus a tenfold increase in saccharogenic activity resulting from the addition of papain was associated with an increase in gas production of only about 4%. The data certainly suggest that saccharogenic activity has only a very minor effect on gassing power. However, the evidence is of little value because it seems probable that, in the determination of free gassing power, not only the free beta-amylase, but also that part of the latent beta-amylase liberated by the proteolytic enzymes of the flour, are active in the dough.

The correlation between free and total gassing power was 0.97. In these circumstances it does not seem necessary to record the data for the total gassing power of the individual varieties. The data for the customary determination of free gassing power are given in Table II and discussed in the next section.

Saccharogenic Activities and Gassing Power. Data for the saccharogenic activity of the malt flour, and for those parts of it attributed to beta- and alpha-amylase, are given in the left-hand section of Table II. The varieties are listed in order of decreasing saccharogenic activity. Considerable differences exist between the varieties; with respect to each property, the highest value is more than twice as great as the lowest value.

It is interesting to note that Garnet, No. 1, is not only highest in alpha-amylase activity and gas stimulation, but is also highest in beta-amylase activity. Although it is not easy to see it in the data, there is a loose but significant association between beta- and alpha-amylase activities. The correlation is 0.49, which exceeds the 5% level of significance but falls short of the 1% level, 0.51. It appears that in wheat as in barley (Sallans and Anderson, 1939) there is a slight tendency for varieties which are high in beta-amylase to be high in alpha-amylase also. The association is very loose, and has little practical significance.²

The data in Table II show that approximately 10% of the saccharogenic activity of the malt flour was due to the alpha-amylase. It thus appears that for most practical purposes the determination of saccharogenic activity may well serve as an adequate measure of comparative beta-amylase activities of wheat malts. In the present study the saccharogenic activity of the malt flour and beta-amylase activity gave a correlation coefficient of 0.996. This fact is worth

² Kneen, Beckord, and Sandstedt (1941), in commenting on this matter, write: "Sallans and Anderson (1939) also found a significant correlation between malt-saccharifying and malt-liquefying activities. The data in Table III are not in agreement with this finding." However, a footnote on the same page shows that the correlation coefficient for "the data in Table III" is significant; it is 0.603, which is above the 5% level, 0.576.

recording if only to show that a very high correlation between two properties does not necessarily show that one is dependent entirely on the other, and that a fundamental causal relation does not exist with any additional factor or factors, in this case alpha-amylase activity.

The total saccharogenic activity (papain) of the wheat flour is fairly closely correlated with the saccharogenic activity of the malt flour; the correlation coefficient is 0.88. As would be expected it is more closely correlated with the beta activity of the malt flour for which the correlation coefficient is 0.90. It follows that the comparative saccharogenic activities, and more especially the beta activities, of malt flours made from different varieties can be estimated with moderate accuracy from data on the total saccharogenic activities of the unmalted wheat flours.

Data on total Lintner values (papain) of the wheat flour are also included in Table II. Since these and total saccharogenic activities (papain) are determined by methods which differ mainly in the temperatures of diastasis, it is not surprising that a correlation of 0.97 is obtained. For comparative purposes the methods are essentially interchangeable.

The last column of data in Table II deals with the gas production of the wheat flour. Here again there are appreciable differences between the varieties; the highest gives a value of 301, and the lowest gives 217. Gas production in the wheat flour is not correlated with any property other than the alpha-amylase activity of the malted wheat flour. The correlation is 0.54 which, though low, exceeds the 1% level of significance. Thus it is not likely to be fortuitous. The explanation may be that gas production in the unmalted wheat flour is related to alpha-amylase activity in the same product, and that the latter is related to alpha-amylase activity in the malted wheat flour. Unfortunately this hypothesis cannot be examined as determinations of the alpha-amylase activity of the unmalted flour were not made.

Summary

Samples of 23 varieties grown at 16 stations in Canada were composited to provide sets of samples representing each variety and each station. The samples were milled, and were also malted and then milled. Properties of the wheat flours and malted wheat flours were examined.

Increases in the gassing power of a standard base flour, resulting from additions of 0.2% of malted wheat flour, varied between 130 and 163 ml depending on the variety, and between 140 and 160 ml depending on the station. Corresponding variations in alpha-dextrinogenic activity were 32 to 83 units for varieties, and 37 to 57 units for stations.

The intervarietal correlation coefficient for these two properties was 0.71, and the interstation correlation was 0.79.

Additional data are reported for the varietal composites only. A very loose association, represented by a correlation of 0.49, was found between alpha- and beta-amylase activities of the malt flour. About one-tenth of the saccharogenic activity of the malts was contributed by the alpha-amylase. Determinations of total (papain) saccharogenic activity and total (papain) Lintner value in the wheat flour are interchangeable; the correlation is 0.97. The total (papain) saccharogenic activity of the wheat flour is correlated with the saccharogenic activity of the malted wheat flour (0.88), and is, as would be expected, more highly correlated with the beta-saccharogenic activity of the malted wheat flour (0.90).

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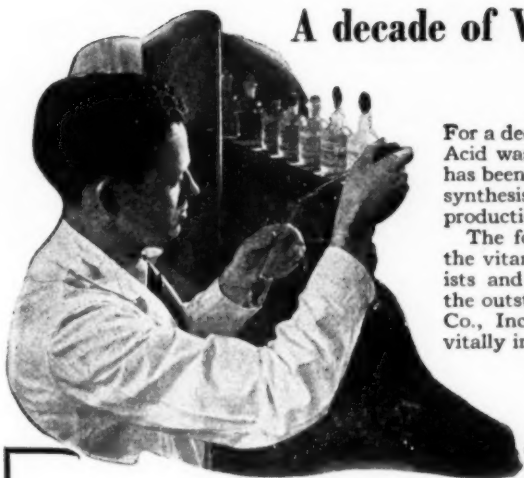
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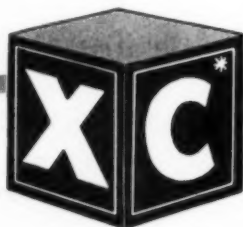
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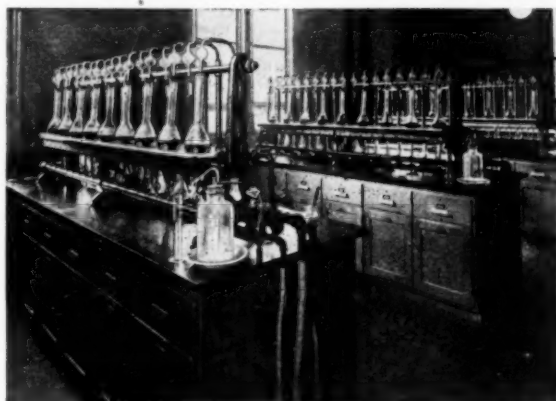


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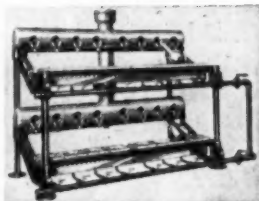
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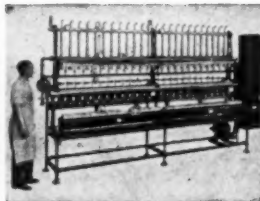
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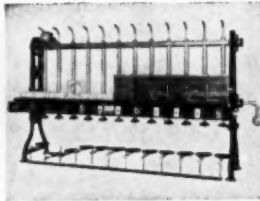
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The unfortunate tendency of most iron compounds to accelerate the development of rancidity in cereals is common knowledge. These iron compounds tend to dissociate and yield free, uncombined iron which causes trouble and promotes off odors and flavors.

Laboratory research has revealed certain facts which provide an interesting explanation for the *striking difference* in behavior between such iron compounds and Sodium Iron Pyrophosphate. In Sodium Iron Pyrophosphate the iron is anionic . . . even in solution the iron remains firmly bound to the phosphate radical in such a way as to render it trouble-free.

Since November 1940, literally hundreds of keeping tests in connection with the flour enrichment program have been made with Victor Sodium Iron Pyrophosphate. In these tests, as well as in practical use, no sound flour has ever become rancid because of the presence of Sodium Iron Pyrophosphate . . . yes, even when 10 times the required amount of iron was added and

the sample exposed to extreme conditions of humidity and heat.

Today, the addition of iron to flour, cereals, and similar staple foods is generally accepted as desirable from a nutritional point-of-view. Send today for liberal experimental sample of Victor Sodium Iron Pyrophosphate. Make your own keeping tests . . . see for yourself that Sodium Iron Pyrophosphate is the safe iron for enriching your product.

VICTOR Mineral Supplements

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Dicalcium PHOSPHATE
Tricalcium PHOSPHATE
Calcium PYROPHOSPHATE
Sodium Iron PYROPHOSPHATE
Ferric ORTHOPHOSPHATE
Ferric PYROPHOSPHATE

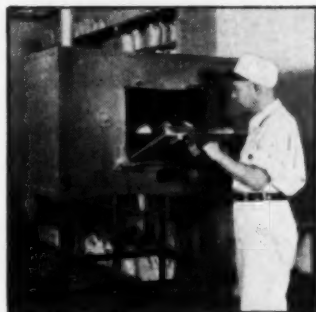
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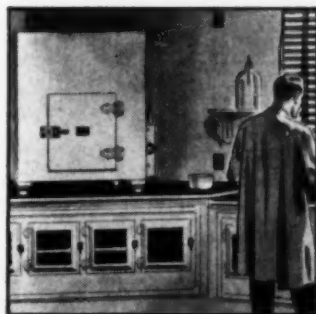




ROTARY HEARTH OVEN (special No. 150). Standard sizes handle up to 48 pup loaves or 12 one-lb. loaves. Efficient, smooth-running hearth; standard diameters to 38" with special models up to 108"



STATIONARY HEARTH OVEN (No. 139). Bakes 10 to 216 pound-loaves at a time, depending on unit size. Standard section dimensions (inside) from 11"x11"x9" to 57"x60"x9". Thermostatic controls.



LABORATORY TEST OVEN (No. V-15). Ideal for rapid, accurate moisture tests. Forced draft; $\pm 1^\circ\text{C}$. accuracy. Range to 500 F. (260° C.). Inside sizes: 13"x13"x13" to 37"x25"x37".

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To you who attend the Convention and to those unable to come, we are pleased to announce that Despatch ovens *are available again*.

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